Optimization of antipsoriatic treatments with special reference to monitoring of Methotrexate-induced liver toxicity

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Optimization of antipsoriatic treatments with special reference to monitoring of Methotrexate-induced liver toxicity

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"Take each man's censure, but reserve thy judgment."
William Shakespeare

Voor Erik, mijn ouders, Saskia en Dick

Abbreviations

DNA:	Desoxy-ribonucleic-acid
EMEA:	European Medicines Agency
GRE:	Glucocorticoid Response Element
ICAM-1:	Intercellular Adhesion Molecule-1 (CD54)
LFA-1:	Leucocyte function-associated antigen-1 (=CD11a/CD18)
LFA-3:	Leucocyte function-associated antigen-3 (=CD58)
MTX:	Methotrexate
NHG:	Nederlands Huisartsen Genootschap
PASI:	Psoriasis Area and Severity Index
PASI-50:	Percentage of patients achieving 50% or more reduction in PASI
PASI-75:	Percentage of patients achieving 75% or more reduction in PASI
PIIINP:	Aminoterminal propeptide of procollagen type III
PUVA:	Psoralen and Ultra Violet A
RAR:	Retinoid Acid Receptor
Th1:	T helper 1
Th2:	T helper 2
TNF:	Tumor Necrosis Factor
TNFR:	Tumor Necrosis Factor Receptor
UVB:	Ultra Violet B
VDR:	Vitamin D Receptor
VDRE:	Vitamin D Receptor Element

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Chapter

General Introduction

1.1 Focus of this thesis

Psoriasis is a chronic and incurable disease with an average onset during the second decade of life. This means that many patients are challenged to manage their disease for decades.

In the Netherlands two guidelines are available for the management of patients with psoriasis: the 'NHG standaard', which focuses on topical treatments and which has been created by the general practitioners and the guideline 'photo(chemo)therapy and systemic therapy' which is the guideline of the Dutch Society of Dermatology and Venereology[1-3].

Based on these guidelines but also based on the information available in international reviews[4,5] it is evident that a 'stepwise approach', starting with topical therapy (1), followed by photo(chemo)therapy and classical systemic therapies (2), followed by biologics (3) is the 'life of therapies'. The risk of toxicity increases with each sequential treatment in the stepwise approach. Various treatment approaches, like rotation, combination, sequential and intermittent therapy are used to reduce the risk of toxicity.

The focus of the present thesis was to optimize the treatment of psoriasis, reconciling this treatment paradigm and to carry out a study in each of these 3 phases of intervention.

Step1: In order to reduce the number of patients exposed to the potential side effects of systemic treatments and biologics, improvement of efficacy safety ratio of topical treatments with excellent safety profile is important. We have focussed on the combination of the vitamin D_3 analogue calcipotriol and the corticosteroid betamethasone dipropionate as an improvement in the topical treatment of psoriasis. A study was performed to find out the effect of the combination and of both monotherapies on psoriasis relevant T cells, in order to understand the rationale of this successful combination (chapter 2).

Step 2: Of the treatments photo(chemo)therapy and classical systemic treatments, methotrexate is a major treatment, which permits long-term safe control with an impressive efficacy. Of concern is liver toxicity. Monitoring this hepatotoxicity is dealt with in the Guideline of the Dutch Society for Dermatology and Venereology. However, amongst dermatologists, rheumatologists and gastroenterologists there is a major discussion whether monitoring of the liver with regular liver biopsies is needed. Chapter 3 addresses this question.

Step 3: Despite optimization of topical and classical systemic treatments and various treatment approaches, the available topical, photo(chemo) therapy and classical systemic therapies may not provide adequate continuous long-term disease control in all patients. In such cases, the biologicals may be able to provide a more consistent

control of symptoms with a good safety profile. Biologicals are indicated for patients with severe psoriasis who have failed all classical treatments. Early 2005 the biologicals etanercept and efalizumab became available for routine treatment of a high-need psoriasis population. In chapter 4 the results of both biologicals in a new unit for routine care of these high need patients are described.

In chapter 5 the observations in chapters 2-4 will be integrated and recommendations for adaptations of the existing guidelines will be presented and perspectives for future development will be provided.

Definition

Psoriasis is a chronic recurring non-infectious, inflammatory disease that can affect skin, nails and/or joints.

1.2 Epidemiology and clinical presentation of psoriasis

Psoriasis is a common disease, affecting 1,5-3% of the world's population. There are racial variations with a high prevalence in western populations while the disease has not been observed in Native Americans, Eskimo's and Saami. Men and women are equally affected. The disease can occur at any age although two peak incidences have been reported: a peak between 16 and 22 years and a later one at 57-60 years[4,6-9].

There are different disease variants of psoriasis with different cutaneous manifestations: combinations of these different forms are possible. The most common type is psoriasis vulgaris (chronic plaque psoriasis) which accounts for approximately 90% of all cases[4-6,9].

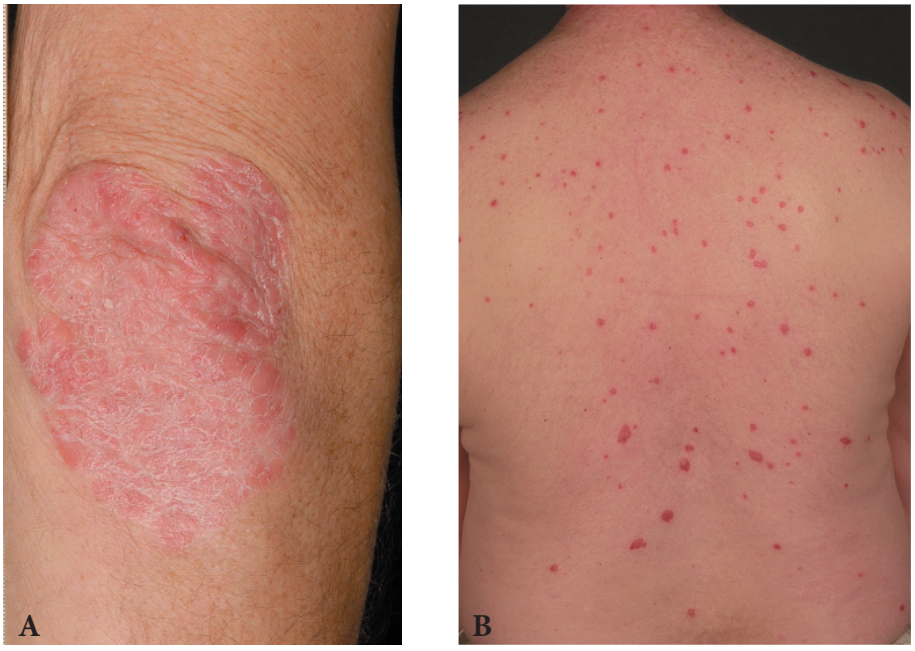

Figure 1: *Clinical presentation of psoriasis vulgaris with sharply demarcated erythematosquamous plaques (A) and guttate psoriasis with droplike erythematosquamous plaques on the skin (B).*

Chronic plaque psoriasis is characterized by, often symmetrically distributed, sharply demarcated erythematosquamous plaques of varying size with predilection sites on the dorsal sites of the elbows, knees and on the scalp and sacral region. The scales of these lesions are typically silvery white and scratching these looks like scratching on a wax candle ("signe de la tache de bougie"). The course is chronic with exacerbations and remissions[4,5]. **(figure 1)**

In guttate psoriasis (psoriasis gutatta) many erythematous and scaly droplet-like lesions are scattered diffusely over the body. It develops particularly after streptococcal infections and often clears in a few weeks to months[4,5]. Sterile pustules and deep erythematous areas characterize pustular psoriasis. **(figure 2)** The pustules can be localized (pustulosis palmoplantaris) or generalized[4,5].

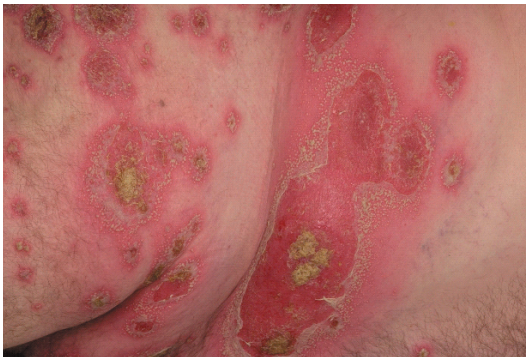

Figure 2:
Clinical picture of a generalized form of pustular psoriasis with deep-red erythematous areas and pustules.

Flexural psoriasis (psoriasis inversa) appears as non-scaly inflamed patches in the skin folds, particularly in the axillae, groins, submammary and peri-anal region and retro-auricular folds[4,5].

In erythrodermic psoriasis all or almost the entire cutaneous surface is involved and patients may become ill and febrile and develop hypothermia, dehydration and protein loss requiring hospitalization[4,5].

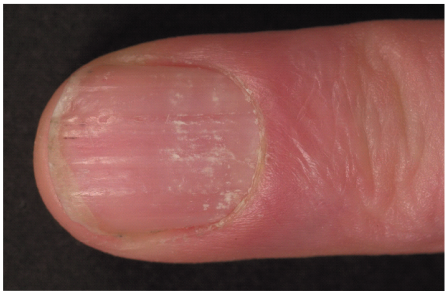

Figure 3:
Clinical picture of a psoriatic involvement of the nail with typical pitting of the nail plate.

Extracutaneous manifestations of psoriasis are nail psoriasis (psoriasis unguium) and psoriatic arthritis. Nail involvement is present in 25-50% of all types of psoriasis and more frequently with psoriatic arthropathy. The most common changes seen in the nails are pitting, discolouration (oil drop phenomenon), subungual hyperkeratosis and onycholysis[4,5]. **(figure 3)** Psoriatic arthritis occurs in about 5-10% of the patients although this percentage is discussed: up to 39% has been suggested in the recent questionnaire by Europso[4,5,10].
The studies in this thesis have been concentrating on chronic plaque psoriasis.

1.3 Aetiology and pathogenesis of psoriasis

Psoriasis seems to be a multifactorial disease caused by the interaction between genes and environmental triggering factors[4,5].

Several gene loci have been linked to psoriasis. It has been hypothesized that polymorphisms, both in genes involved in immune function and in genes involved in keratinocyte biology be responsible for psoriasis[5,11].

Triggering factors may play a role in the initiation of the disease process and exacerbation of pre-existing disease. Triggering factors that have been reported are trauma (Koebner phenomenon), infections, drugs (β-blockers, lithium, antimalarials), endocrine factors (pregnancy), psychogenic stress, increased alcohol consumption, obesity, smoking and HIV[4,5].

Histologically the disease is characterized by epidermal hyperproliferation with loss of differentiation, dilatation and proliferation of dermal blood vessels and accumulation of inflammatory cells (particularly neutrophils and T lymphocytes)[5]. **(figure 4)**

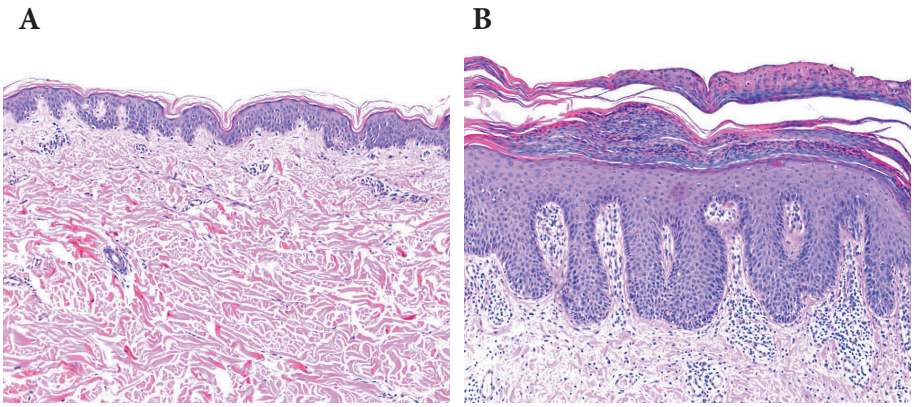

Figure 4: *Histology of normal (A) and involved psoriatic skin (B)*

1.4 Available treatments for psoriasis

Psoriasis is still an incurable disease, relapsing after cessation of any therapy. Treatment should be individualized and the decision to employ a particular treatment should depend on age, personality, profession, general health, intelligence and resources, quality of life, type, extent, duration and natural history of psoriasis.

Medications with the least potential toxicity are preferred and medications with significant toxicity are preferentially restricted to those patients with a physically, socially, economically or emotionally disabling and therapy resistant psoriasis. Outpatient topical therapy

should be the first step in case of a stable psoriasis, causing as little as possible disturbance in a patient's routine. If topical treatment fails, the next step should be photo(chemo)therapy (UVB, PUVA). The third step involves treatment with one of the classic systemics: methotrexate, cyclosporin, retinoids, fumaric acids. If all treatments have failed, the last step is treatment with the so called biologics[1-3,5].
For more extensive reviews on antipsoriatic treatments the reader is referred to the literature.

1.4.1 Topical treatments

This section describes the current topical treatments for chronic plaque psoriasis: Emollients and keratolytics, Coal tar, Dithranol, Corticosteroids, Vitamin D_3 derivatives, Calcipotriol/betamethasone dipropionate, Calcineurin inhibitors and Topical retinoids.

Emollients and keratolytics
Emollients contain no pharmaceutically active components and are used to hydrate and soften dry skin. They can be used both in lesional and unaffected psoriatic skin. Keratolytics are prescribed to reduce the thick scale of psoriatic plaques to enhance the penetration of topical medications and phototherapy[12].

Coal tar
Coal tar has been used in topical therapy for more than a century. It has anti-inflammatory, and also antimitotic effects, modulating the itch and clinical severity of the psoriatic lesions. It can also be used in conjunction with UV therapy[4,5].

Dithranol (anthralin)
Dithranol induces a cascade of free radicals in the skin, which reduces proliferation and inflammation. Patients can be treated with twenty-four-hours application, which is highly effective but time consuming, or short-contact application, being both easier and more convenient for the patient. Because of habituation in case of repeated application, the concentration of dithranol should be cautiously increased with a maximum of 3 times a week, starting with 0,05 or 0,1%[4,5,13,14].

Corticosteroids
Corticosteroids have been used for decades in mild to moderate psoriasis. On the face and neck, flexures and genitalia mild corticosteroids are the first-line therapy because other topical treatments can induce irritation and potent corticosteroids induce atrophy at these sites. Corticosteroids can be given as monotherapy or in combination with other topical therapies[5,15-18].

Corticosteroids bind to an intra-cellular glucocorticoid-receptor. This complex binds to a nuclear glucocorticoid response element

(GRE) resulting in anti-inflammatory, immunosuppressive and anti-mitotic effects of these drugs[19]. Corticosteroids are manufactured in various vehicles (ointments, creams, lotions, foams and gels) with different efficacy, ointments being most effective[20].

To organize the available corticosteroids they have been classified in class I to IV, class I being low potent, class II being potent and class III and IV being super potent corticosteroids[20].

Vitamin D_3 derivatives

Vitamin D_3 derivatives are the first-line treatment, as monotherapy or in combination, in mild to moderate psoriasis. Because of their lipophilic character Vitamin D_3 derivatives pass the cell membranes easily and finally bind to nuclear Vitamin D Receptors (VDR). This complex binds to specific DNA sequences (Vitamin D response elements (VDRE)) in the promoter region of certain vitamin D target genes that are involved in epidermal proliferation, inflammation and keratinisation[4,5,21-27].

At the moment there are three synthetic vitamin D_3 derivatives available for topical treatment of psoriasis in Europe: calcitriol (Silkis®), calcipotriol (Daivonex®) and tacalcitol (Curatoderm®). These topical agents are manufactured as ointment, cream or lotion[20].

Calcineurin inhibitors

Calcineurin inhibitors block calcineurin, an important enzyme involved in the calcium dependant signal transduction in T-cells, thereby inhibiting the transcription and synthesis of various cytokines[28]. There are two different preparations: Tacrolimus ointment 0,03% or 0,1% (Protopic®) and Pimecrolimus cream 1% (Elidel®)[20]. Calcineurin inhibitors are only an effective treatment for intertriginious and facial forms of psoriasis[29,30].

Topical retinoid: Tazarotene

Tazarotene is a synthetic acetylenic retinoid (0,05% or 0,1%) and available as gel and cream. It has been shown to have anti-proliferative and anti-inflammatory activities and as such has a modest antipsoriatic effect[31]. It has not been registered in the Netherlands.

Combination treatments

It is always of great importance to optimize the available treatments by improving efficacy and reducing side effects. One way to accomplish this might be by combining existing treatments. To increase efficacy and reduce side effects, combination treatment is advised for most of the patients. Coal tar and dithranol can be combined with any antipsoriatic treatment. However, there are few evidence-based studies.

Tazarotene combined with topical steroids and UVB as been proven to be more effective and safe as compared to mono therapies. The fixed combination of calcipotriol/betamethasone dipropionate

(Dovobet®) has been proven to be more effective than twice-daily calcipotriol or bethamethasone diproprionate mono therapy[15,17,32-35]. This two-compound product contains calcipotriol 50µg/g and betamethasone dipropionate 0,5mg/g[20].

1.4.2 Photo(chemo)therapy and classical systemic therapy

Photo(chemo)therapy

If topical treatment is ineffective or in case of moderate to severe psoriasis with a large affected body area the next step is often phototherapy. Available forms are broadband UVB (290-320nm), narrowband UVB (311 +/- 2nm) and PUVA photochemotherapy. UV irradiation is thought to exert immunosuppressant effects, and the induction of DNA damage and apoptosis in hyperproliferative keratinocytes[4].

Nowadays, narrowband UVB is the most optimal irradiation available with three times weekly appearing the optimum regimen for efficacy. It has been found to be superior to conventional broadband UVB in psoriasis, producing longer remissions with lesser side effects. In addition, narrowband UVB is more convenient and also probably less carcinogenic than PUVA therapy, although the effectiveness appears to be similar to each other. An interesting development is home narrowband UVB treatment. PUVA irradiation consists of UVA plus topical or systemic psoralens. PUVA is given 2-4 times weekly[4,36,37].

Because long-term treatment with PUVA has serious carcinogenic hazards, PUVA should only be considered as a primary treatment in elderly patients in whom psoriasis covers at least 20% of the body surface, and who cannot be controlled by conventional topical therapy. In younger patients or in patients with less widespread psoriasis, other considerations may justify therapy according to individual circumstances[4].

Combination treatment of PUVA and retinoids is also currently used to treat psoriasis[4,36,37].

Classical systemic therapies

This section describes the current systemic treatments for psoriasis: methotrexate, cyclosporin, systemic retinoids and fumarates.

Methotrexate (MTX)

MTX is indicated for patients with severe psoriasis when topical treatment and photo(chemo)therapy have been shown to be ineffective or contraindicated[2,3].

As a folic acid antagonist it competitively inhibits dihydrofolate reductase, an enzyme necessary for DNA synthesis. In addition it has important effects on pyrimidine and purine metabolism, resulting in anti-inflammatory effects, inhibition of T-cell mediated immune responses and abnormal rapid epidermal cell proliferation[38,39].

MTX is administrated as oral treatment or, less frequently, as

subcutaneous, intramuscular or intravenous treatment. Administration can be in a single weekly dosage or divided in 3 doses over a 24-hour period each week (Weinstein schedule). The maximum weekly single dosage, advised by the Dutch guideline, is 22,5 mg weekly[2-4].

Cyclosporin
Cyclosporin is a calcineurin inhibitor inhibiting the transcription and synthesis of various cytokines[4,20]. Such as MTX it is a first-line systemic treatment for severe psoriasis after conventional therapies (topical treatment and/or photo(chemo)therapy) are ineffective or inappropriate[2,3]. The advised daily dosage by the guideline of the Dutch Society for Dermatology and Venereology is 3-5mg/kg, divided in a twice daily dosage[2-4].

Systemic retinoids:acitretin
"Retinoid" is the family name of natural and synthetic analogues of vitamin A. These vitamin A analogues bind to the nuclear retinoid acid receptor (RAR). This complex, again, binds response elements at DNA level, resulting in anti-inflammatory and anti-proliferative effects[4,5].

Monotherapy with acitretin is effective in 30% of patients with chronic plaque psoriasis, and in erythrodermic and pustular psoriasis. Combination therapy with UVB and retinoids is possible[4,5].

Treatment of psoriasis vulgaris with retinoids in combination with another therapy (topical corticosteroids, dithranol, tar, selective UVB phototherapy and PUVA), enhances therapeutic effectiveness and allows a lower retinoid dosage. Acitretin (Neotigason®) is the most prescribed retinoid[4-6].

Fumarates (*Fumaderm*®)
Fumaderm has not been registered in the Netherlands. Registration has been obtained in Germany, France and Switzerland. Data about the safety of fumarates are limited. The exact mode of action is unknown but there is some evidence that fumarates restore the Th1 and Th2 cytokine balance[4].

1.4.3 Biological therapies

Many patients are challenged to manage their disease for decades. However, the suitability of classical systemic treatments as continuous long-term maintenance therapy is questionable and despite the efforts to optimize available treatments by various approaches (combination, rotation, sequential, intermittent) in some 'high-need' patients, disease control is insufficient. In those 'high-need' patients, the biologicals may be able to provide a more consistent control of symptoms with conservation of a good safety profile.

Since 2004 the European Union has approved three biologic agents for the treatment of adult patients with moderate to severe

plaque psoriasis who failed to respond to, or have a contraindication to, or are intolerant of other systemic therapies. These biologic agents are: etanercept (Enbrel®), efalizumab (Raptiva®) and infliximab (Remicade®). The registration of a fourth biological, adalimumab (Humira®) is expected to occur at the end of 2007. In the United States alefacept has been registered since 2004 for treatment of psoriasis. Short-term data suggest that these agents are safe and mainly mild adverse events have been reported so far. However, long-term safety data over many years are still largely unknown.

There are two major targets of the biologicals available so far: TNF-α (etanercept, adalimumab and infliximab) and T-cells (efalizumab, alefacept).

Anti-TNF-α agents

TNF-α

TNF-α is a cytokine mainly produced by macrophages but also by a variety of other cells such as lymphoid cells, mast cells, endothelial cells and fibroblasts. It promotes an inflammatory response that in turn induces many of the clinical problems of psoriasis. There are two distinct TNF Receptors (TNFR): a soluble form and a cell surface receptor[40].

Etanercept

Etanercept is a fusion protein produced by recombinant DNA technology. This fusion protein consists of the extra-cellular ligand-binding portion of the human tumour necrosis factor receptor and the Fc portion of human IgG1. It is a soluble form of the TNF receptor and can bind to two soluble TNF molecules thereby blocking their interaction with cell surface TNF-α receptors[41-43].

Etanercept is administrated subcutaneously 25 or 50 mg twice weekly. The European Agency for the Evaluation of Medicinal Products (EMEA) label advises to start on 50 mg etanercept twice weekly the first 3 months and to continue treatment with a reduced dosage of 25 mg biweekly[41-46].

At week 12, 34% and 49% of patients receiving etanercept 25mg biweekly and 50 mg twice weekly, respectively, a reduction of at least 75% in pre-treatment PASI score (PASI-75) was achieved. At the same time point and dose levels, 58% and 74%, respectively, achieved PASI-50[41-46].

Studies have shown that response is maintained after this dosage reduction and is not followed by deterioration in disease control. Forty nine percent of patients reached a PASI-75 at week 12 on 50mg of etanercept biweekly, while 54% of patients achieved a PASI-75 at week 24 following a dosage reduction for a further 12 weeks to 25mg etanercept biweekly[41-46].

If PASI improvement is less than 50% after 3 months, etaner-

cept should be discontinued. Also, due to limited data on the safety of continued treatment, a maximum duration of 24 weeks of treatment has been advised by the EMEA. Retreatment, however is possible.

Infliximab
Infliximab (Remicade®) is a chimeric (combination of mouse and human components) monoclonal antibody. It binds both membrane-bound and soluble forms of TNF-α.

Infliximab administration is by intravenous infusion, 5mg/kg at week 0, 2, 6 followed by 2 month intervals with good results: 88% of patients reaching a PASI-75 and 97% of patients reaching a PASI-50 by or before week 10 have been reported[42-46].

Adalimumab
Adalimumab is a fully human IgG1 monoclonal antibody produced by recombinant DNA technology in a mammalian cell expression system and binds specifically to TNF-α. As a consequence the interaction between TNF-α and its two distinct receptors is blocked. Adalimumab should also be administrated subcutaneously. It has not been registered for psoriasis yet but registration is expected soon.

The results of a phase II, double-blind, placebo-controlled study in 149 adults patients with moderate-severe psoriasis showed a PASI-75 improvement in 77% of the patients receiving 40 mg subcutaneously weekly and in 67% of the patients receiving 40 mg subcutaneously every other week at week 24. In another recent study in which patients were treated with adalimumab 40 mg subcutaneously every other week, 67% and 56% showed a PASI-50 and PASI-75, respectively, after 12 weeks[42,43,45,47].

Anti-T-cell agent

Efalizumab
Efalizumab (Raptiva®) is an immunosuppressive, recombinant humanized monoclonal IgG antibody. It binds to the T-cell surface molecule CD11a, the α-subunit of leucocyte function-associated antigen-1 (LFA-1). This in turn inhibits the interaction between LFA-1 and intracellular adhesion molecule 1 (ICAM-1) thereby inhibiting T-cell activation, trafficking of T-cells from the circulation into the skin and reactivation of T-cells.

Efalizumab is administered by a subcutaneous injection once a week with an initial dosage of 0.7mg/kg followed by 1.0mg/kg once a week. Treatment with efalizumab should only be started in patients with stable chronic plaque psoriasis.

About 27% and 59% of patients reach a PASI-75 and PASI-50, respectively at 3 months of therapy. However, PASI response rates have proven to continue to improve from weeks 12-24 with 44% and 67% reaching a PASI-75 and PASI-50, respectively after 24 weeks of continuous treatment[42,43,45,46,48].

Alefacept (*Amevive*®)
Alefacept is, like etanercept a fusion protein, produced by recombinant DNA technology. It consists of the extracellular CD2-binding portion of the human leukocyte function antigen-3 (LFA-3) linked to the Fc portion of human IgG1. Alefacept inhibits activation of CD2, CD4 and CD8 positive T-cells, which stimulate hyperproliferation of keratinocytes.

Possible routes of administration are intravenously or intramuscularly with a standard dosage regimen of weekly application of 7,5 mg iv or 15mg im for 12 weeks. Alefacept has been registered in Canada, the United States, Australia, Switzerland and Israel for the treatment of adult patients with moderate to severe psoriasis who are candidates for systemic treatment[4,42,43].
So far, it has not been approved by the EMEA.

1.5 Limitations of the available anti-psoriatic treatments

All treatments have limitations with respect to efficacy, safety and tolerability.

1.5.1 Limitations of topical treatments

Coal tar
Immediate limitations of tar include messiness, unpleasant smell and staining, irritant, allergic and phototoxic responses and folliculitis. As tar has a well established carcinogenic potential, long-term toxicity of coal tar products is a concern[4,5].

Dithranol
Short-term side effects include irritation and staining of the skin, nails and clothes, which is a serious limitation of this time honoured principle[4,5].

Corticosteroids
According to the guideline of the Dutch Society of Dermatology and Venereology, in adults to prevent the development of side effects, class II and III topical corticosteroids should be applied with a maximum of 100 grams weekly and class IV corticosteroids with a maximum of 50 grams weekly[1,2,20].

Possible side effects are: skin atrophy, striae, teleangiectasia, vascular dilatation, purpura, hypertrichosis, acneiform eruptions and hypopigmentation. If patients use more than the maximally allowed quotations of class III or class IV corticosteroids patients are at risk for suppression of the pituitary-adrenal axis[4,5,20].

Vitamin D_3 derivatives
Vitamin D_3 analogues have a limited toxicity but cause skin irritation in up to 30% of the patients and disturbance in calcium metabolism when more than 100 grams of calcipotriol ointment/cream/week or 210 grams of calcitriol ointment a week is used[4,5,20].

Calcineurin inhibitors
Long-term safety data on calcineurin inhibitors are not available yet. Well-known immediate side effects are burning sensation at the site of application, erythema, irritation, hyperesthesia, paresthesia, folliculitis and pain. The efficacy is limited to flexural and facial psoriasis[20].

Topical retinoid: tazarotene
Side effects observed using tazarotene are irritation, burning, pruritus, erythema and desquamation within and peripheral to the lesion where this has been applied. To avoid teratogenic side effects of topical tazarotene, no more than 20% of the body surface should be treated with it[31].

1.5.2 Limitations of photo(chemo)therapy

The most common short-term side effects of photo(chemo)therapy are erythema and sunburn and also pruritus in case of PUVA therapy. Of most concern is the long-term risk of carcinogenicity[4].

1.5.3 Limitations of systemic treatments

Methotrexate (MTX)
Common side effects of MTX are gastro-intestinal symptoms, nausea and abnormal liver function tests. Other known side effects are myelosuppression inducing leukopenia, thrombocytopenia and rarely, folate-deficient megaloblastic anaemia[20,49]. Based on studies and clinical experience, prescription of folate supplementation is advised for every patient receiving MTX[2,3,50].

Hepatotoxicity, including liver fibrosis and cirrhosis, is a major concern and restricts use of MTX in psoriasis. Early studies showed a very high prevalence of up to 50% liver fibrosis and up to 20% liver cirrhosis in psoriatic patients using MTX[51]. However, recent literature suggests that MTX-associated liver damage is less prevalent than previously assumed[38,49,52,53].

Concerns about this hepatotoxic injury have led to dermatologic guidelines that stipulate monitoring patients treated with MTX by liver histology periodically after every 1500 mg cumulative dose. Until now liver biopsy is considered the gold standard method to assess the histological changes, but its application is complicated by some limitations. A liver biopsy is an invasive procedure and can be a burden to the patient. Complications occur in 1-2% of the patients including

pain, localized bleeding, less often pneumothorax, haemothorax, bile peritonitis, haemobilia, and inadvertent puncture of the kidney or intestine. Another limitation of the liver biopsy is the possible chance for sampling error[49,54,55].
Fear for developing liver injury and the liver biopsy procedure make the dermatologist and the patient more reluctant to choose MTX as a treatment alternative.

Cyclosporin
The most important side effects of cyclosporin are dose-related and sometimes irreversible nephrotoxicity and hypertension. These side effects demand regular monitoring of blood pressure, renal function tests and treatment for only several-month courses (maximally one year). Other side effects include increased risk of cutaneous and systemic malignancy (especially in psoriasis patients previously treated with high-dose UV irradiation), tremor, altered liver function tests and gastrointestinal intolerance, elevated potassium and uric acid levels, decreased serum magnesium, mild normochromic normocytic anaemia, gum hyperplasia, hypertrichosis, acral paraesthesia or hyperaesthesia[4].

Systemic retinoids: acitretin
The most concerning side effect is its teratogenicity demanding women to avoid pregnancy within 2 years following treatment with acitretin. Furthermore, extraossal hyperostosis and hyperlipidaemia are a serious concern[4].

Fumarates
Known side effects of the fumarates are renal impairment, flushing, gastrointestinal disturbances, headache and fatigue. Here also, regular blood analysis of liver, kidney and haematological parameters is advised[4].

1.5.4 Limitation of biological therapies

Anti-TNF-α
Most common side effects of anti-TNF-α are injection side reactions and mild infections. Nevertheless, allergic reactions to etanercept, an increased risk for developing serious and especially opportunistic infections like tuberculosis, aggravation of an existing heart failure, demyelinating conditions and malignancies all have been reported during the treatment with anti-TNF-α agents[41-43,45].

Anti-T-cell agent
In case of efalizumab first dose reactions are common including headache, fever, nausea, chills, myalgia within two days following the first two injections. Furthermore, patients might be at increased risk for

developing infections. In case of instable chronic plaque psoriasis, worsening of the psoriasis has often been seen. Other serious adverse events that have been reported during the treatment with efalizumab were malignancies, thrombocytopenia, arthritis and haemolytic anemia[38,42,43,45,56].

1.6 Aims of this thesis

The studies carried out in this thesis are an attempt to optimize the available antipsoriatic treatments with respect to safe disease control.

Aim I: An increased efficacy/safety ratio of topical treatments will decrease the population of psoriatic patients requiring systemic treatments. Clinical evidence is available that the combination of vitamin D_3 and corticosteroids is the most effective principle for topical treatment. Formulations of both compounds are available which are highly acceptable to the patient for use in daily life.
A study was planned **to understand the improved efficacy of a combination therapy of calcipotriol and betamethasone dipropionate in terms of effects on T-cell subsets and epidermal proliferation and differentiation.**

Aim II: A limitation of the classical systemic treatments for psoriasis is the cumulative toxicity potential. Methotrexate is the first line systemic treatment according to the guidelines of the Dutch Society for Dermatology and Venereology. Hepatotoxicity is an important hazard of this treatment.
We will set out a study **to define the degree of methotrexate-induced hepatoxicity and to develop new strategies for monitoring this hepatotoxicity.**

Aim III: Biologicals have been introduced in the treatment of psoriasis in 2005. A prospective evaluation was carried out with respect to the introduction of routine care with biologicals.
In order to evaluate the efficacy and safety of biologicals in routine care, a special unit and a database were set up. High-need psoriasis patients were treated at this unit with biologicals to provide best possible care.

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2
Chapter

An improvement in the topical treatment of psoriasis

This chapter was based on the following publication:

W.H.P.M. Vissers, M. Berends, L. Muys, P.E.J. van Erp, E.M.G.J. de Jong, P.C.M. van de Kerkhof
The effect of the combination of calcipotriol and betamethasone dipropionate versus both monotherapies on epidermal proliferation, keratinization and T-cell subsets in chronic plaque psoriasis. *Experimental Dermatology 2004: 13: 106-112*

An improvement in the topical treatment of psoriasis

Classical topical treatments remain to be the first line approach in the management of psoriasis. In fact 60% of the population of psoriatic patients has mild-moderate disease. Improvement of the efficacy/safety ratio of topical treatments implies that systemic treatments and biologicals more and more are restricted to patients with severe disease.
Various clinical studies have shown that the combination of calcipotriol and betamethasone dipropionate is a major improvement with increased efficacy beyond efficacy of monotherapies. The question remains, however, what the mechanistic rationale for this improved efficacy is.
We set out a study to understand the improved efficacy of a combination therapy of calcipotriol and betamethasone dipropionate in terms of effects on T-cell subsets and epidermal proliferation and differentiation.

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The effect of the combination of calcipotriol and betamethasone dipropionate versus both monotherapies on epidermal proliferation, keratinization and T-cell subsets in chronic plaque psoriasis

Vissers WHPM, Berends M, Muys L, van Erp PEJ, de Jong EMGJ, van de Kerkhof PCM. The effect of the combination of calcipotriol and betamethasone dipropionate versus both monotherapies on epidermal proliferation, keratinization and T-cell subsets in chronic plaque psoriasis. Exp Dermatol 2004: 13: 106–112. # Blackwell Munksgaard, 2004

Abstract: Several reports have indicated that the combination of calcipotriol ointment and potent or ultrapotent corticosteroids are more effective and better tolerated, as compared to the monotherapies.

The aim of the present study was to find out the effect of combination of calcipotriol ointment once daily and betamethasone dipropionate ointment once daily vs. the effect of twice-daily applications of each of the two treatments as monotherapy during a four-week treatment period.

Seven patients with chronic plaque psoriasis were included for treatment with the three treatment schedules. Biopsies were taken before treatment and after four weeks of treatment, and markers for epidermal proliferation (Ki-67) and epidermal differentiation (keratin-10) were studied using a quantitative image analysis, and T-cell subsets in epidermis and dermis (CD4, CD8, CD25, CD45RO, CD45RA, CD94, CD161, and CD2) were studied using immunohistochemical scoring.

The most impressive clinical result was reached with the combination. Calcipotriol proved to have a major effect on the proliferation marker Ki-67 and differentiation marker keratin-10, whereas the effect on T-cell subsets was more selective with major reductions of $CD45RO^+$ and $CD8^+$ T cells. In contrast, the effect of betamethasone dipropionate on the epidermis was restricted to a normalization of differentiation with a highly significant increase of keratin-10 positive epidermal surface without a significant effect on Ki-67 positive nuclei, and the effect on T-cell subsets was restricted to a reduction of natural killer T-cell receptors designated by CD94 and CD161 in the epidermis. The combination of the two treatments did not affect the proliferation marker Ki-67 and keratinization marker keratin-10, beyond the effect of calcipotriol monotherapy. However, the combination had a profound effect on, virtually, all T-cell subsets, beyond the effect of the monotherapies.

It is concluded that the action spectra of calcipotriol and betamethasone on the psoriatic plaque are different and that the combination has effects on T-cell subsets, beyond the addition of the effects of monotherapies.

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Key words: immunohistochemistry – psoriasis – T-lymphocytes – topical treatment

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Introduction

Topical treatment with calcipotriol as monotherapy or in combination with a potent or ultrapotent topical corticosteroid has become the first-line treatment of psoriasis. The combination has been shown to be more effective and safe, as compared to monotherapies (1–3).

However, so far, few reports are available on the mode of action of combination of calcipotriol with a topical corticosteroid vs. calcipotriol monotherapy and corticosteroid monotherapy.

The psoriatic lesion is characterized by epidermal proliferation (increased recruitment of cycling epidermal cells), abnormal keratinization with a decreased compartment of normally differentiated cells (keratin-10-positive epidermal surface), and inflammation with a prominent role of T cells, in particular activated memory effector T-cells (4).

The effect of vitamin D3 analogs and corticosteroids on aspects of epidermal proliferation and keratinization as well as some T-cell subsets have been studied (5–12). However, the conclusions from these studies are at variance. Furthermore, the effect of the combination therapy on epidermal proliferation and keratinization as well as T-cell subsets has not yet been investigated by immunohistochemistry.

Therefore, a within-subject study of three treatments was initiated to compare:

1. The combination of calcipotriol ointment (5 mg/g) once daily and betamethasone dipropionate (0.64 mg/g) ointment once daily.
2. Calcipotriol (5 mg/g) ointment twice daily.
3. Betamethasone dipropionate (0.64 mg/g) ointment twice daily.

The treatments were carried out during 4 weeks, and a punch biopsy was taken before and after treatment from the test lesions treated with the three schedules.

One limitation of past studies was the lack of quantification and uniformity in the assessment of immunohistochemical results. Recently, quantitative image analysis provided new possibilities for more reproducible scoring of immunohistochemical results. To study epidermal proliferation and differentiation, we used quantitative image analysis.

The following features were studied:

1. Epidermal proliferationwas assessed by counting the Ki-67-positive nuclei per mm basement membrane by quantitative image analysis, which characterizes those epidermal cells which have escaped from the resting Go population. In previous studies, it has been shown that this phenomenon is crucial to epidermal proliferation in psoriasis (13).
2. Epidermal differentiation was studied by measuring the keratin-10-positive epidermal surface. In the psoriatic plaque, the epidermal cell layers expressing keratin-10 are reduced.
3. The T-cell is of major pathogenic significance. Recently, specific T-cell-targeted treatments have been shown to improve psoriasis. CD4þ T-cells dominate in early psoriasis and $CD8^{+}$ T-cells in chronic plaques (14). In particular, activated T-cells (CD2 and CD25) belonging to the memory effector subset (CD45RO) have been shown to play a major role (4). Natural killer (NK) cells belonging to innate immunity have been suggested to be of relevance to psoriasis. The NK cell receptors on T-cells (CD94 and CD161) have been suggested to represent important cross talks between innate immunity and acquired immunity (4,15,16).

Methods

In our study, seven patients participated with stable plaque psoriasis with an extent of at least 5%. They did not receive any systemic treatment for at least 4 weeks and no topical therapy for about 2 weeks before the start of the study. All patients were able to understand care instructions.

They were asked to apply betamethasone dipropionate ointment two times a day to the psoriatic lesions at the right side of the body, the combination of betamethasone dipropionate ointment once a day and calcipotriol ointment once a day to the lesions at the left side of the body, and calcipotriol ointment twice a day to an arbitrary chosen lesion for 4weeks. After having applied the ointment of one treatment schedule, the patient was asked towash his/her hands before applying the ointment for the other treatment schedule. Before and after treatment, the target lesions were observed and scored for the degree of erythema, desquamation, and induration. The score may range from slight (1) to very severe (4). The total score of erythema, desquamation, and induration, the sum score, was used for clinical assessment.

Biopsies were taken from the three target lesions corresponding to the three different topical treatments. This was performed before and after treatment.

Biopsies were embedded in Tissue Tek OCT compound (Miles Scientific, Naperville, IL, USA), snap frozen in liquid nitrogen, and stored at 80C until use.

Sections were sliced to 6 mm thick and were air-dried for 30min. Then, the sections were fixed in cold acetone for 10min. After blocking for 5 min for endogenous peroxidase, using 0.2% sodium azide, they were washed in phosphate-buffered saline (PBS) for 10 min. Subsequently, sections were incubated with the primary antibodies for 1 h. The following primary antibodies (mouse anti-human) were used, diluted in 1% bovine serum albumin (Sigma, St Louis, MO, USA)/PBS: anti-CD2 (clone MT910) (1:50), anti-CD4 (clone MT310) (1:25), anti-CD8 (clone DK25) (1:25), anti-CD45RO (clone UCHL1) (1:25), anti-CD45RA (clone 4KB5) (1:25), anti-CD94 (clone HP-3D9) (1:25), anti-CD25 (clone ACT-1) (1:25), Ki-67 (clone MIB-1) (1:100) (all obtained from DAKO, Copenhagen, Denmark), keratine-10 (clone RKSE60) (1:100) (Monosan Laboratories, Uden, The Netherlands), and anti-CD161 (clone 191B8) (1:25) (Immunotech,Marseille, France).

Sections were washed in PBS for 15 min. Secondary immunoglobulin-G-labeled polymer, horseradish peroxidase (antimouse),

was added for 30min (Envision-kit: DAKO). The sections were washed for 15min in PBS. To visualize the staining, we used 3-amino-9-ethylcarbazol +High Sensitivity SubstrateChromogen for 10min (DAKO).Counterstaining was performed with Mayer's Haematoxylin (Sigma). The sectionswerewashed in tap water anddried. Sections were finally mounted in glycerol gelatin (Sigma). Furthermore, from each patient, we performed a hematoxylin–eosin staining. After dehydration in alcohol and histosafe (Histosafe,Adamas Instrumenten bv., Leersum, the Netherlands) these sections were mounted in Permount.

Immunohistochemical scoring

Quantification of the number of cells positive for CD2, CD4, CD8, CD25, CD45RO, CD45RA, CD94, and CD161 was performed. At 200 x magnification, positive cells in the epidermis were counted from the basement membrane up to the stratum corneum across the whole section. Cells in the dermis were counted from the basement membrane down to 100 mm under the basement membrane, also, across the whole section. Quantification was performed in the unit: positive cells per mm length.

Quantitative image analysis

For K-10, digital photographs were made at 50 x magnification, and for Ki-67, digital photographs were made at 100 x magnification. Each photograph was analyzed using IP-lab software. For quantification of the number of cells positive for K10, we used the following procedure: After choosing a representative 'region of interest' (ROI), all positive segments in the ROI were marked with a color and counted. We subtracted the present dermal surface in the ROI. The ROI is chosen in the center of the section. Quantification was measured as a percentage of ROI as unit. For Ki-67, a line following the stratum basale was drawn after again choosing a ROI, and all positive cells above this line were counted. Quantification was performed in the unit: positive cells per mm length of basement membrane.

Statistical analysis

We used a two-sided paired t-test to compare the number of positive cells, and percentage of K-10-positive epidermal surface before and after treatment. P<0.05 was considered as statistically significant. P<0.01 was considered as highly statistically significant.

Results

For all three treatment modalities, there was a significant decrease in sum score. The mean percentage decrease in sum score was 71% for the combination therapy, 66% for betamethasone dipropionate, and 67% for calcipotriol. Reduction of the sum score for combination therapy gave the smallest P-value (combination: $P=6.57 \times 10^{-7}$) (calcipotriol: $P=9.5 \times 10^{-6}$) (betamethasone dipropionate: $P=2.97 \times 10^{-5}$).

There was a statistically significant decrease in the number of Ki-67-positive cells (proliferating keratinocytes) for the combination treatment (P<0.01) and monotherapy with calcipotriol (P<0.001). (Fig. 1) There was no statistically significant decrease of Ki-67-positive keratinocytes per mm basement membrane after monotherapy with betamethasone dipropionate. Concerning differentiation, there was a statistically significant increase of the keratin-10-positive epidermal surface after all three treatment modalities. For the two monotherapies, there was a highly significant (P<0.001) increase of keratin-10-positive epidermal-surface. (Fig. 2) For the combination therapy, the increase was also significant (P<0.05).

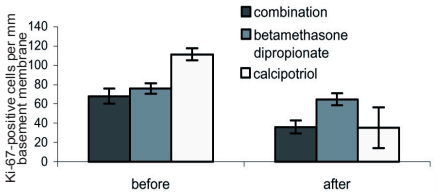

Figure 1. Ki-67 positive keratinocytes per mm basement membrane before and after treatment with combination therapy (p <0.01), betamethasone dipropionate 2dd (no statistical significance) and calcipotriol 2dd (p<0.001). (Mean ± SEM)

All T-cell subsets decreased during treatment with the combination therapy (Fig. 3), betamethasone dipropionate ointment (Fig. 4), and calcipotriol ointment (Fig. 5).

The combination resulted in a highly significant reduction of $CD8^+$, $CD2^+$, and $CD25^+$ T-cells in the epidermis, and $CD2^+$, $CD25^+$, and $CD94^+$ T-cells in the dermis (P<0.01). $CD4^+$, $CD45RO^+$, and $CD45RA^+$T-cells showed a significant reduction in dermis and epidermis (P<0.05). $CD161^+$ T-cells reduced significantly exclusively in the dermis (P<0.05). No significant reduction was shown for $CD94^+$ and $CD161^+$ T-cells in the epidermis. Betamethasone dipropionate ointment did not result in a highly significant reduction of T-cell subsets. Apart from a significant reduction of $CD94^+$ and $CD161^+$ T-cells in the epidermis

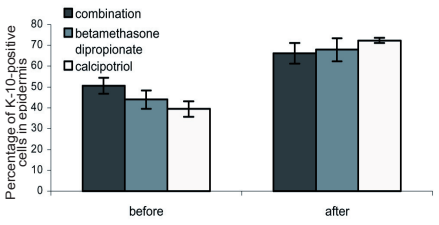

Figure 2. Percentage K10 positive epidermal surface before and after treatment with combination therapy (p<0.05), betamethasone dipropionate (p <0.001) and calcipotriol (p<0.001). (Mean ± SEM)

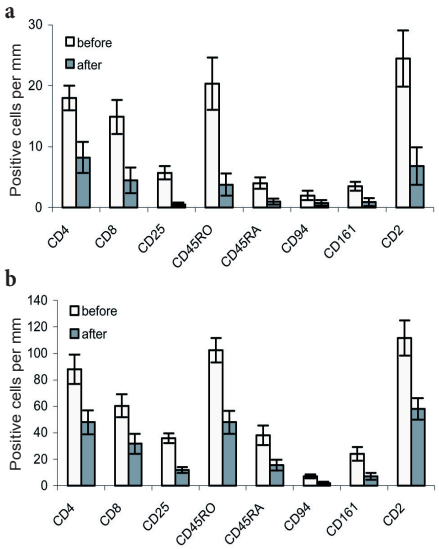

Figure 3. T-Lymphocytes in the epidermis (a) and dermis (b) before and after combination therapy. (Mean ± SEM)

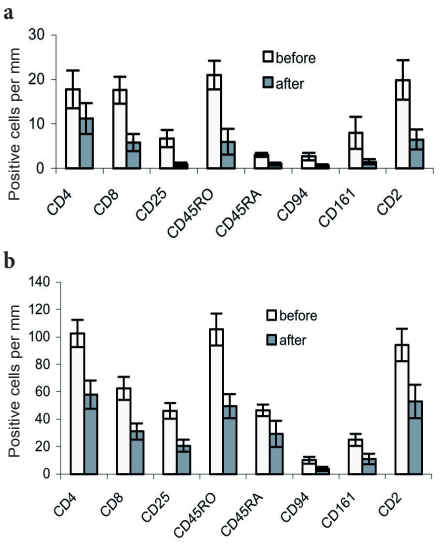

Figure 5. T-Lymphocytes in the epidermis (a) and dermis (b) before and after treatment with calcipotriol. (Mean ± SEM)

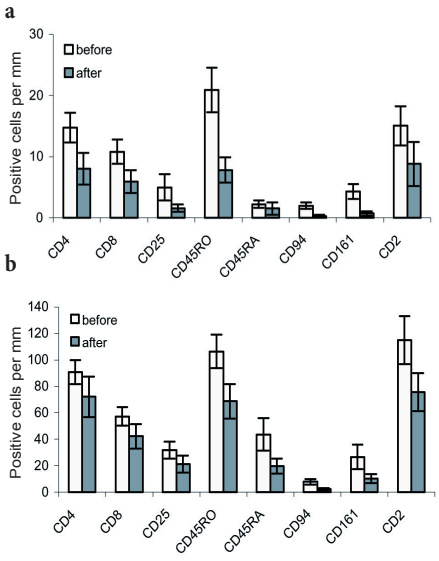

Figure 4. T-Lymphocytes in the epidermis (a) and dermis (b) before and after treatment with betamethasone dipropionate. (Mean ± SEM)

(P<0.05), the reduction of T-cell subsets in dermis and epidermis did not reach the level of statistical significance. Calcipotriol ointment caused a highly significant reduction of CD45RO$^+$ T-cells in dermis and epidermis, and a highly significant reduction of CD8$^+$ T-cells in the dermis (P<0.01). CD8$^+$, CD25$^+$, CD45RA$^+$, CD94$^+$, and CD2$^+$ T-cells in the epidermis, and CD4$^+$, CD25$^+$, CD94$^+$, and CD161$^+$ T-cells in the dermis reached a statistically significant reduction (P<0.05). CD4$^+$ and CD161$^+$ T-cells in the epidermis, and CD45RA$^+$ and CD2$^+$ T-cells in the dermis did not show a statistically significant reduction.

In Fig. 6, the percentage decrease of T-cell subsets has been shown. It can be seen that all T-cell subsets show a more impressive reduction, following calcipotriol, as compared to betamethasone dipropionate, both in dermis and epidermis, with exception of CD4$^+$, CD94$^+$, CD161$^+$, T-cells in the epidermis (Fig. 6a) and CD45RA$^+$, CD94$^+$, and CD161$^+$ T-cells in the dermis (Fig. 6b). In the epidermis, the reductions were more expressed, following treatment with the combination with the exception of CD94$^+$ and CD161$^+$ T-cells. In the dermis, all T-cell subsets showed the most impressive reduction, following treatment with the combination, although the reduction of CD4$^+$, CD8$^+$, and CD45RO$^+$ were approached by the reduction after calcipotriol ointment only.

Discussion

In the present investigation, using a four-week treatment period, the combination of calcipotriol and betamethasone dipropionate ointment, and monotherapy with betamethasone dipropionate ointment and calcipotriol ointment resulted in asubstantial and statistically significant reduction of the sum score. Al-

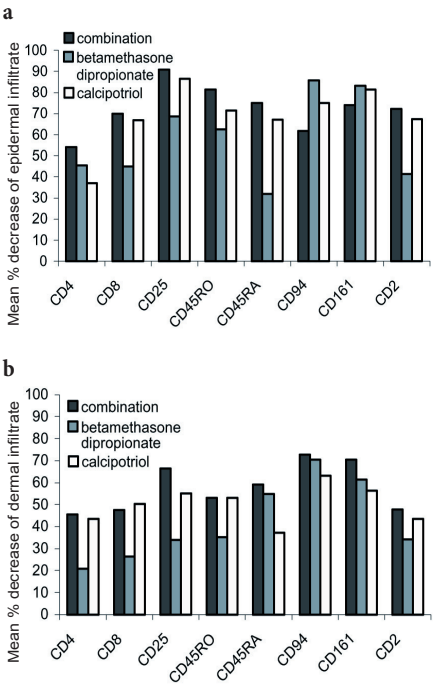

Figure 6. Mean percentage decrease of epidermal infiltrate (a) and dermal infiltrate (b) for the three different topical treatments.

though no statistically significant difference could be shown between the three treatments, the most significant improvement was reached by the combination. This observation reconfirms several observations by other groups that the combination is more effective, as compared to monotherapy with either calcipotriol ointment or a potent topical corticosteroid(1–3,17–20).

Calcipotriol proved to induce a highly significant inhibition of epidermal proliferation and to cause a highly significant increase of keratin-10-positive epidermal surface (Figs 1 and 2). In previous studies, it was shown that Ki-67-positive nuclei decreased (1,12,21), keratin-10-positive cell layers increased (10), involucrin-positive cells increased (1,21), and keratin-16 expression decreased (12,21, 22), during calcipotriol treatment. Although some authors have suggested that calcipotriol has a preferential effect on epidermal proliferation and keratinization (11,12,23), other authors have suggested that the effect on T-cells is relevant to the mode of action of calcipotriol (1,7,10).

Reduction of $CD3^+$, $CD45RO^+$, and $CD4^+$ T-cells (7,10,21), reduction of $CD8^+$ T-cells (10), reduction of $CD2^+$ T-cells (1,9,12), and reduction of $HLA\text{-}DR^+$ epidermal cells by vitamin D3 analogs have been reported (8). One group failed to show a reduction of $CD45RO^+$ T-cells by calcipotriol (11). In the present study, calcipotriol proved to have a highly significant reduction of $CD45RO^+$ T-cells in dermis and epidermis, and a highly significant reduction of $CD8^+$ T-cells in the dermis as outstanding features. Although most T-cell subsets showed a statistically significant reduction during calcipotriol treatment, $CD4^+$ and $CD161^+$ T-cells in the epidermis showed a reduction below the level of statistical significance, and, also, in the dermis, the slight reductions of $CD45RA^+$ and $CD2^+$ T-cells were not statistically significant. These observations suggest that calcipotriol has a substantial effect on epidermal proliferation and keratinization, whereas the effect on T-cell subsets is more selective with a major effect on $CD45RO^+$ and $CD8^+$ T-cells. In this respect, it is of importance that $CD45RO^+$ T-cells, i.e. the memory effector cells, constitute a pathogenically relevant T-cell subset and that selective apoptosis of these cells by alefacept results in improvement of psoriasis (24–26). $CD8^+$ T-cells have been shown to be the dominating T-cell population in chronic plaque psoriasis, whereas $CD4^+$ T-cells are more common in early psoriatic lesions (14).

Treatment with betamethasone dipropionate did not result in a statistically significant reduction in the number of Ki-67-positive keratinocytes, although the increase in the percentage of keratin- 10-positive epidermal surface was highly significant. Previous studies have shown substantial reductions of keratin-16 and keratin-10-positive cells during treatment with potent and ultrapotent topical corticosteroids, approximating the reduction of calcipotriol (10,23). Previous studies on the assessment of Ki-67-positive nuclei during treatment with topical corticosteroids revealed that ultrapotent topical corticosteroidsmay have a substantial effect on Ki-67-positive nuclei (13,27). It can be concluded that betamethasone dipropionate results in a substantial change in compartmentalization of the psoriatic epidermis without a significant effect on the recruitment of cycling epidermal cells as assessed by Ki-67 expression. The effect of topical corticosteroids on T-cells has been studied before. De Jong et al. did not show a significant effect on $CD2^+$ T-cells, following treatment with the ultrapotent topical corticosteroid budesonide (13). In the present study, the effect of betamethasone dipropionate on T-cell subsets showed, exclusively, a statistically significant reduction of $CD94^+$ and $CD161^+$ T-cells in the epidermis. CD94 and CD161 are NK T-cell receptors and may play an important role in the cross talk between innate immunity and acquired immunity (4,15,16). Activation of NK T-cell receptors may inhibit cytotoxic T-cells to release T helper type-1 cytokines (15). This inhibition is lost when NK T-cell receptors are

reduced. Therefore, the significant reduction of NK T-cell receptors in the epidermis by betamethasone dipropionate might be an explanation for the tachyphylaxis.

From the profile of actions on epidermal proliferation, keratinization, and T-cell subsets, it is attractive to speculate that calcipotriol and betamethasone dipropionate are complementary. The highly significant effect of calcipotriol on epidermal proliferation vs. the limited effect of betamethasone dipropionate is an example in this respect. The substantial effect of calcipotriol on all T-cell subsets except $CD4^+$ and $CD161^+$ T-cells vs. the pronounced effect of topical corticosteroids on $CD94^+$ and $CD161^+$ T-cells without affecting other T-cell subsets is another example.

The combination of calcipotriol and betamethasone dipropionate resulted in a highly significant reduction of the proliferation marker Ki-67, although the increase of the keratin-10 population was statistically significant but less pronounced as compared with both monotherapies.

It can be concluded that the combination of treatments does not result in an additional reduction of the proliferation marker and increase of the keratinization marker, beyond the reductions by both monotherapies. However, all T-cell subsets, including the $CD4^+$ T-cell population, showed a significant or highly significant reduction in dermis and epidermis, except for the $CD94^+$ and $CD161^+$ populations in the epidermis. Although betamethasone dipropionate showed a preferential reduction of these NKT-cell receptors, in the combination, this aspect seemed to be lost.Comparing the effects of the three treatments (Fig. 6a,b), it can be seen that all T-cell subsets, following treatment with the combination, show the most impressive reductions with the exception of $CD94^+$ and $CD161^+$ T-cells in the epidermis, whereas the reduction of $CD4^+$, $CD8^+$, and $CD45RO^+$ T-cells was approached by the reduction of calcipotriol monotherapy.

In the comparison between calcipotriol ointment and betamethasone dipropionate ointment in combination vs. monotherapies, it should be reconciled that the combination implied a reduction of the frequency of the individual treatment from twice daily (monotherapy) to once daily (combination therapy).

Recently, in some countries, the formulation Dovobet1 (Leopharmaceutical products, Denmark) became available, which is a unique formulation providing optimal bioavailability and stability of the individual active treatments. As twice or once daily principle, this treatment is more effective as compared to both monotherapies (28,29). In a previous study, using flow cytometry, it was shown that this new combination principle causes a major reduction of inflammatory cells, whereas the advantage on epidermal proliferation is limited, as compared to monotherapies (6).

Although the combination of calcipotriol and betamethasone dipropionate has no advantage on epidermal proliferation and keratinization, the broad range of effects on T-cell subsets cannot be explained by a simple addition of effects but rather suggest a synergistic principle of the combination.

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3
Chapter

How to improve monitoring of liver toxicity in patients treated with Methotrexate

This chapter was based on the following publications:

M.A.M. Berends, E.M.G.J. de Jong, P.C.M. van de Kerkhof, M.J.P. Gerritsen
Dermatologists' adherence to the guideline of the Dutch Society of Dermatology and Venereology with respect to the treatment with Methotrexate for severe chronic plaque psoriasis. Results from a Dutch Survey. *Dermatology 2007;215(1):45-52.*

M.A.M.Berends, J.Snoek, P.C.M. van de Kerkhof, E.M.G.J. de Jong, M.G.H. van Oijen, J.P.H.Drenth
Liver injury in long-term Methotrexate treatment in psoriasis is relatively infrequent. *Aliment Pharmacol Ther. 2006 Sep 1;24(5):805-11.*

M.A.M.Berends, J.Snoek, P.C.M. van de Kerkhof, E.M.G.J. de Jong, M.G.H. van Oijen J.P.H.Drenth
Biochemical and biophysical assessment of MTX-induced liver fibrosis in psoriasis patients: Fibrotest predicts presence and Fibroscan predicts absence of significant liver fibrosis. *Liver International 2007 Jun; 27(5): 639-45.*

Maartje A.M.Berends, Martijn G.H. van Oijen, Josje Snoek, Peter C.M. van de Kerkhof, Joost.P.H.Drenth, J. Han. Van Krieken, Elke M.G.J. de Jong
The reliability of the Roenigk classification for liver damage after methothrexate for psoriasis. A clinicopathologic study of 160 liver biopsies. *Arch Dermatology 2007 Dec;143(12):1515-9.*

How to improve monitoring of liver toxicity in patients treated with Methotrexate

Chapter 3 is about MTX hepatotoxicity. As written before, fear for developing liver injury and the liver biopsy procedure make the dermatologist and the patient more reluctant to choose MTX as a treatment alternative. However, MTX has proven to be effective and it will continue to play an important role in the treatment of psoriasis. This, plus the fact that studies vary widely with respect to prevalence of MTX-induced liver injury underline the importance of further research in this area.

First of all a survey was conducted among dermatologists and residents in dermatology to document their adherence to the guideline of the Dutch Society of Dermatology and Venereology with respect to the treatment with MTX for severe chronic plaque psoriasis. The results of this survey, together with the wide variation in prevalence of MTX-induced liver injury in psoriasis found in literature and the known restrictions of a liver biopsy led to another study evaluating the prevalence and development of liver injury in MTX treated psoriasis.

Until now, the gold standard to monitor liver injury is a liver biopsy. Complications of this liver biopsy restrict its clinical use. Therefore, there is a pressing need for non-invasive, reliable alternative methods to monitor MTX-induced liver injury in psoriasis patients. In this thesis a pilot study was carried out, evaluating the accuracy and feasibility of the Fibroscan® and Fibrotest, two novel, non-invasive methods that might be able to assess MTX-induced hepatic fibrosis in psoriasis patients. Despite the results of this and other studies searching for alternatives, the liver biopsy is still inevitable in many cases. Although liver damage is graded according to the Roenigk classification, the Roenigk classification itself is subjective and insensitive to small changes, particularly when assessing fibrosis; lumping all biopsies with more than minimal fibrosis as advanced fibrosis. Furthermore, this classification has never been validated and no literature is known about the inter-observer reliability. Since the assessment of liver damage is essential in the management of psoriasis patients the results of the Roenigk scoring system should be reproducible with little inter-observer error. Therefore a study was performed determining the inter-observer reliability of the Roenigk score as classification system of liver damage and its possible consequences for clinical practice.

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Dermatologists' Adherence to the Guideline of the Dutch Society of Dermatology and Venereology with Respect to the Treatment with Methotrexate for Severe Chronic Plaque Psoriasis: Results from a Dutch Survey

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Key Words
Methotrexate · Guideline, Dutch Society of Dermatology and Venereology · Psoriasis

Abstract
Background: In 2003, the Dutch guideline 'Photo(chemo) therapy and systemic therapy for severe chronic plaque psoriasis' was established. ***Objectives:*** To document how closely this guideline is followed in clinical practice with respect to the methotrexate (MTX) treatment and to formulate recommendations to adjust the guideline. ***Methods:*** A survey was conducted among Dutch dermatologists and residents in dermatology. The questionnaire assessed the knowledge of and the adherence to the guideline with respect to MTX treatment. ***Results:*** Fifty percent of the contacted dermatologists/residents responded. Fifty-two percent follow the guideline with respect to MTX. Liver biopsy and the frequency of blood investigations cause a discrepancy between guideline and reality. There is a lack of consensus between guidelines of the different specialisms concerning liver biopsy. ***Conclusion:*** The need for liver biopsies in combination with the frequent check-ups and the lack of consensus between rheumatologists, hepatologists and dermatologists seem to restrict the adherence to the guideline.

Introduction

Methotrexate (MTX) is an established and relatively inexpensive treatment for severe psoriasis. Concerns about the potential danger of toxicity have prompted the development of guidelines for monitoring patients receiving MTX [1–4].

In 2003, the Dutch guideline 'Photo(chemo)therapy and systemic therapy for severe chronic plaque psoriasis' was established. The aim of this guideline was to summarize the scientific background of current systemic therapies for moderate to severe psoriasis and, based on a systematic literature review, to give evidence-based recommendations for daily practice for Dutch dermatologists. In this way a guideline for the treatment of psoriasis is available for dermatologists, taking into consideration the efficacy, side effects and the burden for patients, in order to give the best care available. The Dutch guideline, as evidence-based guideline, can be regarded as representative of the currently available national guidelines in other countries.

To the Committee Psoriasis of the Dutch Society of Dermatology and Venereology the question raised how closely this guideline is followed in clinical practice while treating patients with MTX. To investigate this, the Department of Dermatology of the Radboud Univer-

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Table 1. MTX in psoriasis; revised guidelines of the American Academy of Dermatology

1 Pre-MTX evaluation a CBC count with differential count b Serum creatinine, blood urea nitrogen, urinalysis, creatinine clearance c AST, ALT, alkaline phosphatase, bilirubin, albumin, hepatitis A, B and C serology d HIV antibody determination in patients at risk for AIDS
2 Early-treatment (2–4 months) liver biopsy should be considered if any of the following risk factors are present to a significant degree: a History of or current excessive alcohol consumption b Persistent abnormal liver chemistry studies c History of liver disease including chronic hepatitis B or C d Family history of inheritable liver disease e Diabetes mellitus f Obesity g History of significant exposure to hepatotoxic drugs or chemicals
3 Continuing laboratory studies a CBC count with differential and platelet count weekly for 2 weeks, biweekly for the next month, then approximately monthly depending on leucocyte count and stability of patient b Blood urea nitrogen and serum creatinine at 3- to 4-month intervals c AST, ALT, alkaline phosphatase and albumin every 4–8 weeks (more frequent liver chemistry monitoring in lieu of initial liver biopsy)
4 More frequent monitoring may be useful in the following circumstances: a During initial treatment b When increasing dose c During episodes of increased risk of raised MTX blood levels (e.g. dehydration, impaired renal function, increased concomitant medications such as NSAIDS)
5 Liver biopsy is recommended after a cumulative MTX dose of approximately 1.5 g and thereafter at 1.0- to 1.5-gram intervals
Adapted from Saporito and Menter [3].

Table 2. MTX in psoriasis: guidelines of the British Association of Dermatologists

1 Pre-MTX evaluation a CBC with differential count b Urea and electrolytes (sodium, potassium, creatinine) c Liver function tests: AST, ALT, alkaline phosphatase, bilirubin, albumin, total protein, hepatitis A, B and C serology d Chest X-ray (unless done within the last 6 months) e PIIINP prior to starting MTX
2 Early-treatment (3–4 months) liver biopsy should be considered if there is a history of pre-existing liver damage
3 Continuing laboratory studies a FBC, urea and electrolytes and liver function tests fortnightly for the first 6 weeks after the last dose change, thereafter monthly until stabilized; monitoring can be reduced further, in discussion with the patient, to 3-monthly if the dose and trend remains stable b PIIINP every 2–3 months
4 Liver biopsy should be considered for patients with a Elevation of pre-treatment PIIINP above 8.0 g/l b Elevation of PIIINP above normal range (1.7–4.2 μg/l) in at least 3 samples over a 12-month period c Elevation of PIIINP above 8.0 μg/l in 2 consecutive samples
PIIINP = Aminoterminal propeptide of procollagen type III.

sity Medical Centre Nijmegen, The Netherlands, in conjunction with the Committee Psoriasis of the Dutch Society of Dermatology and Venereology, conducted a survey among Dutch dermatologists and residents in dermatology.

This publication describes the adherence of the Dutch dermatologist to the guideline 'Photo(chemo)therapy and systemic therapy for severe chronic plaque psoriasis', in relation to the treatment of patients with MTX. These results together with an update of the recent literature may lead to recommendations to adjust the guideline with respect to treatment with MTX. Because of the resemblance to the international guidelines, these results may, at least to some extent, be applicable to other countries.

A summary of the American, British and Dutch guidelines is drawn in tables 1, 2 and 3.

Methods

Questionnaires were sent to 448 Dutch dermatologists and residents. The names were selected from the membership directory of the Dutch Society of Dermatology and Venereology.

Questionnaire

The interview included professional characteristics of the dermatologists such as function (dermatologist or residency programme), years of experience and type of their practice (academic or peripheral hospital). The second part of the survey focused on MTX treatment for psoriasis patients such as the estimated number of patients with psoriasis they treated and whether or not they currently use MTX therapy. Furthermore detailed questions were asked on MTX prescribing such as their considerations regarding the decision to start MTX, baseline and follow-up liver

Table 3. MTX in psoriasis: guidelines of the Dutch Society of Dermatology and Venereology

1	Pre-MTX evaluation
	a CBC with differential count
	b Serum creatinine, urea, urinalysis, creatinine clearance
	c AST, ALT, alkaline phosphatase, G-GT, albumin, bilirubin, LDH, hepatitis B and C serology
	d Pregnancy test
	e Chest X-ray
	f Liver echo
2	Early-treatment liver biopsy should be considered if any of the following risk factors are present to a significant degree:
	a History of or current excessive alcohol consumption
	b Persistent abnormal liver chemistry studies
	c History of liver disease including chronic hepatitis B or C
	d Family history of inheritable liver disease
	e Diabetes mellitus
	f Obesity
	g History of significant exposure to hepatotoxic drugs or chemicals
3	Continuing laboratory studies
	a CBC count with differential and platelet count at weeks 1, 2 and 4 and then every 4–8 weeks
	b Liver function tests: 3 days after starting MTX, thereafter every 4–8 weeks (AST, ALT, AP and albumin)
	c Serum creatinine and creatinine clearance at 3-month intervals; creatinine clearance only in case of special reasons
	d Urinalysis
4	More frequent monitoring may be useful in the following circumstances:
	a When increasing dose
	b During episodes of increased risk of increased MTX blood levels (e.g. dehydration, impaired renal function, increased concomitant medications such as NSAIDS)
5	Liver biopsy
	a Liver biopsy is recommended after a cumulative MTX dose of approximately 1.5 g and thereafter at 1.5-gram intervals

biopsies and blood tests. The last part focused on their opinion about the guideline and their recommendations.

Questions were both open and closed and a combination of these two.

Processing the Questionnaire

To some questions several answers were possible. The answers to the open questions were divided into categories. For example, in case of question 6 the categories were denominated corresponding to the categories of the guideline: severe, therapy-resistant, chronic plaque psoriasis (I1), pustular psoriasis (I2) and erythrodermic psoriasis (I3). Answers which were not (completely) in conformity with the categories in the guideline were assigned to the category I4. A category I5 was established for the remaining answers.

Results

Questionnaires were received for data analysis from 223 dermatologists or residents in dermatology (50% response rate). Six dermatologists were not in clinical practice and were excluded from further analysis. Of the remainder, 179 were completed by dermatologists and 38 by residents in dermatology. Fourteen of them were not involved in treatment of psoriasis patients. Another 40 dermatologists did not currently prescribe MTX (26 of them had prescribed MTX before). Hundred-sixty-three questionnaires were suitable for analysis.

Guideline

Eighty-nine percent of all respondents were aware of the MTX guideline. Ten percent did not know about the existence of this guideline. From 1%, no answer was received.

MTX for Psoriasis

Thirty-nine percent of the respondents prescribe MTX in case of a severe, therapy-resistant, chronic plaque psoriasis (I1), 2% in case of pustular forms of psoriasis (I2) and 1% in case of erythrodermic forms of psoriasis (I3). Sixty-three percent of them reported the use of criteria that approximate but not completely meet those of the guideline (I4; methods) and 31% noticed considerations that have not been mentioned in the guideline (I5), for example psoriatic arthritis, high age, kidney disturbances, compliance and functional complaints.

Three quarters of the dermatologists and residents never prescribe MTX in case of certain risk factors. Usually this is the case with absolute contra-indications but sometimes also in case of relative contra-indications. A small percentage (5%) always prescribes MTX, also when contra-indications are present (fig. 1).

Baseline Biopsies

Most of the respondents (82%) never order a baseline biopsy routinely before starting MTX treatment; 3% always does. Fifteen percent (15%) order a baseline biopsy for patients with risk factors (fig. 2). Risk factors reported are: liver function test abnormalities, alcohol abuse, signs of liver pathology provided by ultrasound or anamnesis, hepatotoxic medication and liver pathology in the past.

Most (59%) of the 29 physicians who always, or in case of risk factors, request a baseline liver biopsy, indicate to do this after 3 months of treatment. The remainder request it before MTX treatment.

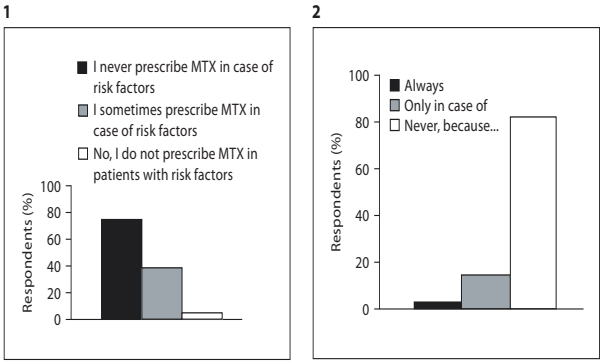

Fig. 1. Do you prescribe MTX in patients with risk factors?
Fig. 2. Baseline biopsy (n = 163)?

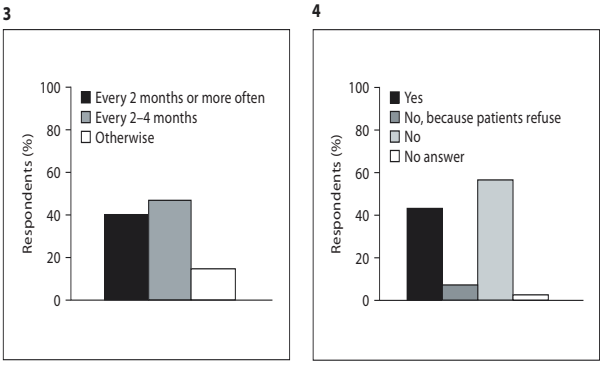

Fig. 3. Frequency of blood monitoring during MTX treatment (n = 163).
Fig. 4. Follow-up biopsies during MTX treatment (n = 163)?

Seventy-two percent of the respondents who let perform a baseline liver biopsy stop or do not start prescribing MTX when an abnormality has been found in the histology, usually in case of Roenigk grade [5] IIIa or IV liver histology according to the Roenigk classification. Others continue prescribing MTX in case of an abnormal liver biopsy but request follow-up liver biopsies more frequently. Ten percent more frequently determine the liver enzymes. Most of them did not mention a Roenigk grade.

Most of the dermatologists and residents (72%) who request a baseline liver biopsy also take into account other factors when drawing conclusions from an abnormal baseline liver biopsy. Some of these are: alcohol intake, diabetes mellitus, obesitas, severity of the psoriasis, possible alternative treatments, the patient's preference, presence of hepatotoxic co-medication, the opinion of the hepatologist, the existence of arthritis and compliance.

Sixty-six percent did not see any life-threatening complications attributable to a baseline liver biopsy. Six physicians did not answer the question at all. The remaining respondents reported life-threatening complications in 1–5% of the patients, attributable to a baseline biopsy.

Regular Monitoring of Blood Count

A great part of the respondents (83%) screen blood, liver enzymes and renal function during MTX treatment (fig. 3).

Follow-Up Biopsies

Forty-four percent order follow-up biopsies during MTX treatment, the remainder does not. Reasons for the latter are: they reduce or withdraw MTX in case of abnormal liver enzymes or a cumulative dosage of 1,5 g. Also, a liver biopsy may have complications or is too demanding for the patient. Other reasons could be: a liver biopsy is not useful according to the rheumatologists, hepatologists refuse to carry them out, or laboratory results and an ultrasound are felt to give enough information (fig. 4).

Just over two thirds of the respondents who request a follow-up biopsy do this after a cumulative dosage be-

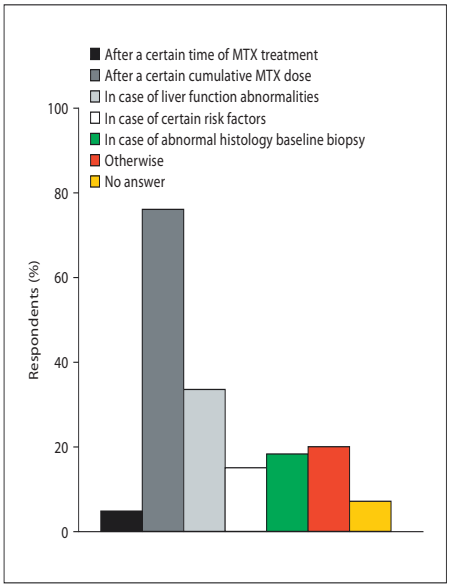

Fig. 5. Reasons for follow-up biopsies.

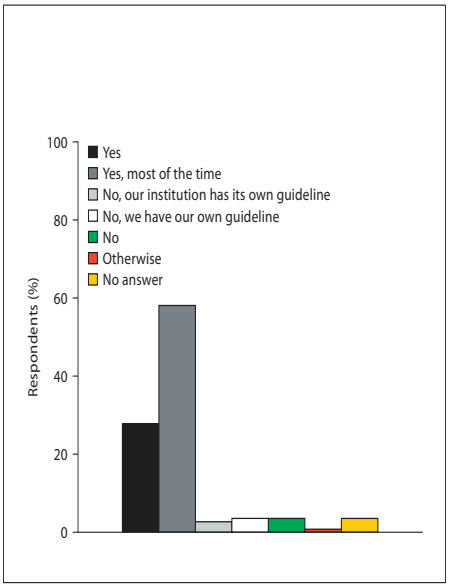

Fig. 7. Is the guideline closely followed with respect to the MTX treatment (n = 147)?

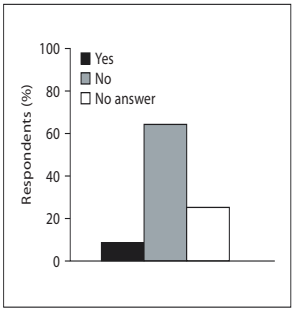

Fig. 6. Continuation of MTX despite contraindications (n = 71)?

tween 1 and 6 g. Others ask for a biopsy in case of abnormal liver function tests. Few respondents order a follow-up biopsy in case of risk factors, aberrant pathological histology of the baseline biopsy or after a period of MTX therapy, an abnormal ultrasound, as well as advice of the hepatologist and/or rheumatologist (fig. 5).

In case of an abnormal follow-up biopsy, 66% of the dermatologists/residents withdraw the MTX treatment. Forty-seven percent of the dermatologists indicate to stop at Roenigk grade IIIb–IV. Thirty-two percent would request liver biopsies more frequently. Most of them (57%) feel to do so at Roenigk IIIa.

When drawing conclusions following an abnormal follow-up liver biopsy, 44% of the respondents take into account the same factors as in the case of a baseline biopsy.

When certain abnormalities in a liver biopsy should lead to withdraw MTX according to the guideline, 10% of the responders might nevertheless continue the treatment occasionally, for example in case of a severe psoriasis with no possible alternative treatment. This will happen in consultation with the hepatologist or in case the patient insists on it. However, the majority never continues MTX treatment in case of related pathology in a liver biopsy (fig. 6).

Guideline

Of those who are aware of the guideline, 52% follow it strictly, except for a liver biopsy. Reasons for this are: possible complications of a liver biopsy, inconvenience for the patient, refusal by the patient, diminished necessity in case of a lower weekly dosage of MTX and lack of

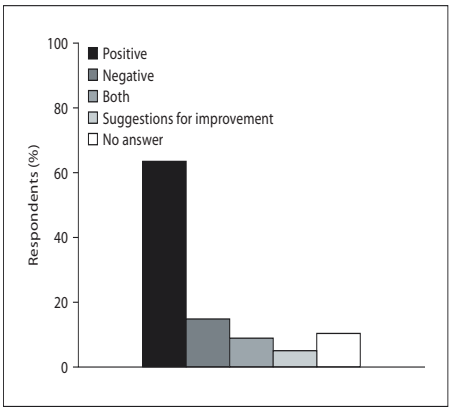

Fig. 8. Opinion about the guideline (n = 147).

concordance with the rheumatologists' and the hepatologists' guideline. A number of dermatologists and residents find the guideline too strict, too defensive and too dogmatic. The check-ups are too frequently scheduled and therefore too intensive for the patient (fig. 7).

Most of the respondents (63%) are positive about the guideline (fig. 8). Thirty-three percent would like to see some improvements. For example, they prefer the guideline to be more practical and simplified, with fewer check-ups. The most frequently given criticism of the guideline is that they would like to see some changes in relation to liver biopsy. For example, a follow-up liver biopsy should not be requested according to a strict schedule. Finally they would like to see more consensus between the hepatologist's, rheumatologist's and dermatologist's management of liver biopsies.

Discussion

The aim of the survey was to investigate how closely the Dutch guideline 'Systemic therapy for severe chronic plaque psoriasis' is followed by Dutch dermatologists in clinical practice during the treatment of psoriasis patients with MTX.

Years ago the Committee Guidelines established the working group 'Psoriasis' and formulated the quality, extent and form of requirements for the guideline 'Photo(chemo)therapy and systemic therapy for severe chronic plaque psoriasis'. Then, during 2 public member meetings of the NVDV (Nederlandse Vereniging voor Dermatologie en Venereologie), the definitive guideline was discussed, accepted in 2003 and finally published in 2003 and 2004 [2, 4]. Besides the published guideline, the guideline can also be found on the website of the NVDV (www.huidarts.info).

The present survey shows that 11% of the dermatologists are not well informed about the guideline. On the other hand, 89% know about the guideline and the majority of them (80%) actually uses it in their clinical practice. However, the need for liver biopsies in combination with the frequent check-ups and the lack of consensus between the different specialists in relation to liver biopsy seem to restrict the adherence to the guideline. The most important reasons to avoid a liver biopsy are: (1) the potential risks of the procedure; (2) the reluctance to prescribe MTX in the presence of (relative) contra-indications; (3) the lack of consensus between the rheumatologists, hepatologists and dermatologists in relation to liver biopsy; (4) the relatively short duration in which MTX is used, and (5) according to some, the opinion that the usefulness of a liver biopsy is not evidence-based.

The generalizability of our findings to all Dutch dermatologists and especially to those in other countries is uncertain. However, MTX retains a central role in the treatment of severe psoriasis in Europe [1] and because of the resemblance to the international guidelines, our results may, at least to some extent, be applicable to other countries.

The reported frequency of liver fibrosis and cirrhosis in liver biopsies of psoriasis patients on MTX treatment varies widely in different studies (fibrosis 1–50% and cirrhosis 0–20%) [6–8]. This uncertainty about a delineated effect led to the recommendation of an intensive monitoring regime [6]. However, MTX-induced liver cirrhosis seems to have a benign character and progresses only slowly [9–11]. Also, a liver biopsy might not be representative because it samples only one 50,000th of the entire liver. The sampling error can approach 30% [12]. Furthermore, liver biopsy is by no means a popular investigation; performing a liver biopsy and histopathological investigation is relatively expensive, patients experience a liver biopsy as inconvenient and the results influence further management only in a few patients [13].

The limited adherence to the guideline in relation to liver biopsy has its roots in the reasons mentioned above. Whereas monitoring liver damage is perceived as very important when using MTX, it appears that the need for a liver biopsy, the gold standard to monitor liver damage, impedes the prescription of MTX. As a consequence it

seems very important to find an alternative for monitoring and detecting liver fibrosis and cirrhosis. This makes it necessary to develop non-invasive methods to detect and monitor liver fibrosis and cirrhosis. It has been shown that fibrosis can develop despite normal serum liver values and normal ultrasound and radioisotope scans. Liver scintigraphy seemed promising as a screening test, but it turned out not to be able to differentiate between the severer forms of liver damage like liver fibrosis and cirrhosis [14]. Two recently published studies show that the values of amino-terminal propeptide of procollagen type III (PIIINP) can be highly sensitive in detecting and monitoring liver fibrosis, although the serum PIIINP value is not organ-specific and may also be raised in children, adolescents and in various pathological states including inflammatory arthritis, scarring, scleroderma, myocardial infarction and hyperthyroidism [12–14]. According to those studies liver biopsies can be avoided when serial PIIINP measurements (once per 3 months) are normal. In case of abnormal PIIINP values without other conditions associated with elevated PIIINP levels, a liver biopsy should be performed [13, 14]. Performing a liver biopsy only in patients with an abnormal PIIINP resulted, according to the study of Maurice et al. [14], in a 45% reduction of all liver biopsies and the study of Chalmers et al. [13] in a sevenfold reduction, without important liver damage being missed. Boffa et al. [15] and Zachariae et al. [8, 16, 17] also concluded that the chance to develop substantial liver fibrosis and the risks of missing significant liver damage are minimal in case of persisting normal PIIINP values.

Of course, a limitation of our study is the fact that selection bias may have affected our results; dermatologists interested in psoriasis and MTX were more likely to participate in this survey. However, the response rate to our survey (49,8%) was at least as good as for similar surveys among dermatologists [1, 18, 19]. Furthermore, 91% of the respondents currently prescribed MTX for psoriasis patients and 80% of them said to use the guideline in their clinical practice. These high numbers make the meaning of the results clearer. Furthermore, reimbursement of the expensive biologicals is restricted to 'high-need' psoriasis patients who may be defined using criteria that include disease severity and resistance and/or intolerance to a number of conventional therapies [18], like MTX. Also, in the future MTX will more and more be used in combination with one of these biologicals. This will strengthen the central role of MTX in the treatment of severe psoriasis in Europe.

Conclusion

In conclusion, we would like to propose the following: it would be preferable to simplify the check-up schedule and make it more uniform for use, to achieve consensus between dermatologists, rheumatologists and hepatologists in relation to liver biopsy, to limit the number of liver biopsies by introduction of measurement of the serum PIIINP value and finally find an alternative for the liver biopsy. Because of the resemblance to the international guidelines, these results may, at least to some extent, be applicable to other countries. Of course, a critical investigation is needed to find out if a simplification of the check-up schedule is safe and advisable.

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Alimentary Pharmacology & Therapeutics

Liver injury in long-term methotrexate treatment in psoriasis is relatively infrequent

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SUMMARY

Background
Methotrexate-induced liver damage in psoriasis has led to dermatologic guidelines that stipulate monitoring of liver injury by means of serial liver biopsies. Recent literature data suggest that methotrexate may be considerably less hepatotoxic than previously assumed.

Aim
To evaluate prevalence and development of liver injury in methotrexate treated psoriasis.

Methods
Retrospective chart review (1976–2005).

Results
Hundred and twenty-five patients (F58/M67; mean age 45.0, SD 12.7 years) received a median cumulative methotrexate dose of 2113 mg (range 180–20 235) over a median period of 228 weeks (range 16–1763). Two hundred and seventy eight liver biopsies were analysed and 71% were classified as Roenigk grade I, 14% as Roenigk II, 14% grade IIIa, 2% grade IIIB and 2% grade IV. Liver injury was not associated with cumulative dose, weekly prescribed dose, age or duration of treatment. Obesity and diabetes were significant risk factors for liver injury. A total of 68 patients had multiple biopsies, 3% improved, 72% did not change and in 25% liver histology deteriorated. The majority of cases (84%) that progressed to Roenigk 2 had a cumulative dose of 1500–6000 mg.

Conclusions
Methotrexate-related liver injury is less frequent than previously thought and mostly occurred at cumulative dose of <6000 mg. Diabetes and being overweight are significantly correlated with liver injury.

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INTRODUCTION

Methotrexate (MTX) is the most commonly prescribed systemic therapy for severe psoriasis. MTX, a folate antagonist, competitively inhibits dihydrofolate reductase, an enzyme necessary for methionine, purine and thymidylate synthesis and, ultimately, DNA synthesis. MTX possesses potent anti-inflammatory effects on T-cell mediated immune responses as it inhibits proliferation or induces apoptosis in activated T-cells and blocks the abnormal rapid epidermal cell proliferation, both responsible for the characteristic skin lesions in psoriasis.[1] Long-term, weekly low-dose methotrexate therapy is associated with some serious adverse reactions as myelosuppression, interstitial pneumonitis and hepatoxicity. The pathogenesis of MTX-induced hepatic damage is poorly understood, but intra hepatocellular accumulation of a polyglutamated metabolite of MTX might be responsible for liver toxic effects. Several studies have suggested that a pre-existent liver disease, overweight, diabetes mellitus (DM) or alcohol use carries an increased risk of hepatotoxicity.[2]

Early studies in MTX treated psoriatic patients reported a very high prevalence of hepatotoxicity with fibrosis occurring in up to 50% and cirrhosis in up to 20%.[3–5] Concerns about liver injury in psoriasis patients has led to dermatologic guidelines that stipulate monitoring periodically after every 1500 mg cumulative dose, in order to identify liver injury by means of serial liver biopsies. Liver biopsy is considered as the gold standard method in the assessment of histological changes,[6] but its application is complicated by some important limitations. A liver biopsy is an invasive procedure and carries inheritent side effects such as pain and localized bleeding.[7, 8] However, recent literature suggests that MTX might be significantly less hepatotoxic and reports that liver fibrosis and cirrhosis are notably less prevalent than previously assumed.[9, 10] These data suggest that reconsideration of our current monitoring strategies for patients on MTX may be warranted. Recent literature proposes that procollagen III aminopeptide (PIIINP) measurements may be of additional value in some patients.[11, 12]

In the present study, we reviewed the clinical details and liver biopsies of 125 patients on long-term, low-dose MTX therapy for psoriasis seen in a large tertiary referral centre from 1976–2005. Our aim was to establish the prevalence of MTX-induced liver injury and to delineate potential contributing factors.

METHODS AND MATERIALS

Subjects

This study is a retrospective chart review over a period between 1976 and 2005. The strategy we followed to identify subjects was as follows. We identified records of all patients who had had a liver biopsy from the pathology files and searched for patients who were known at the Department of Dermatology. Subjects eligible for our study were all psoriasis patients, receiving a weekly dosage MTX and underwent single or serial follow-up liver biopsies for monitoring liver injury.

The clinical records were scrutinized for details regarding age and sex, duration and onset of MTX therapy, cumulative dose at the time of liver biopsy, maximum prescribed weekly dosages, date and reason for MTX-discontinuation, information about the liver biopsy and histological outcomes, presence of risk factors for liver damage (DM, alcohol use and obesity) and other relevant facts from the clinical history. We also recorded laboratory and liver enzyme test results; alanine transaminase (ALAT), aspartate transaminase (ASAT), alkaline phosphatase (AP), γ-glutamyl-transpeptidase (γ-GT), total bilirubin level, platelets and leucocytes counts. Elevated levels were defined as those above the upper limit of normal.

All information was entered in a computerized database. Samples of obtained data were structurally verified to achieve maximum data exactness.

The Department of Dermatology is a large referral centre for psoriasis and we have implemented a monitoring process that closely follows the US guidelines for MTX treatment. Briefly, pre-MTX evaluation includes history and physical examination, laboratory tests consisting of complete blood cell count with differential and platelet count, renal function, liver chemistry and a chest X-ray. In case significant risk factors are absent, a liver biopsy is not necessary until the patient has been treated with 1.5 g of MTX. Monitoring during MTX-treatment includes complete blood cell count with differential and platelet count and liver chemistry every 4–8 weeks, and renal function every 3 months. A follow-up liver biopsy is recommended after every 1.5 g MTX. If there is clinical

suspicion of liver injury the patient should be referred to a hepatologist.[13]

Histology

Percutaneous liver biopsy was performed via right intercostal approach with local lidocaine anaesthesia. The biopsy specimen was immersed in 2% formaldehyde and subsequently fixed with paraffin. Haematoxylin and eosin-stained sections of liver tissue were examined for steatosis, lobular and portal tract inflammation, hepatocyte necrosis and nuclear variability. A von Gieson stain for collagen was assessed for the presence of pericellular and perivenular fibrosis, as well as the expansion of the portal tracts. All liver biopsies, sampled as part of the monitoring process of MTX-induced hepatic injury were, graded according to the Roenigk classification (Grade 1, normal tissue, no/mild fatty change, no/mild nuclear pleomorphism, no fibrosis, mild portal inflammation; Grade 2, moderate/severe fatty changes, moderate/severe nuclear pleomorphism, no fibrosis, moderate/severe portal inflammation; Grade 3a, mild fibrosis, portal fibrotic septa, extending in the lobuli, portal tract enlargement; Grade 3b, moderate/severe fibrosis; Grade 4 = cirrhosis, regenerating noduli and bridging of the portal tracts). We defined a baseline biopsy when it had been performed either at start of MTX treatment and/or before reaching a cumulative dose of 600 mg.

Statistical analysis

Data from the computerized database were analysed using SAS statistical software, version 8.2 (SAS Institute Inc, Cary, NC, USA). Frequency tables were provided for demographic and histological variables. Risk factors for liver injury (presence of DM, overweight and use of alcohol) were analysed over histology, expressed as Roenigk classification and dichotomously analysed using Pearson's chi-square analysis (Fisher's exact test was used where appropriate). The same procedure was used for deviant concentrations of liver enzymes.

Percentage of patients with liver injury, defined as Roenigk classification beyond 1, was plotted per cumulative MTX dose, both for the total patient population and subdivided by the risk factors, mentioned above. Finally, discontinuation of therapy was also analysed per cumulative dose of MTX treatment, using the same type of plots.

RESULTS

Demographics

Hundred twenty-five-psoriasis patients (58 female and 67 male) with an average age of 45 years (s.d. = 13) at start of MTX therapy underwent one or more monitoring liver biopsies. Ninety-two patients carried pre-existent risk factors for MTX induced liver injury at time of the first biopsy. Thirty-nine patients (31%) were overweight (defined as body mass index >25 kg/m^2). Some 49% of patients ($n = 61$) were consuming alcohol, and we categorized 11 (8%) as excessive consumers (>14 U/week). Nine patients (7%) had DM. Twenty-six of them only had a baseline biopsy, fourty-seven had only one or more follow-up biopsies and 19 had both.

The median maximum prescribed weekly dosage MTX was 12.5 mg/week (range 2.5–35 mg/week). Patients received a median cumulative MTX dose of 2113 mg (180–20 235 mg) during a median follow-up period of 228 weeks (range 16–1763 weeks).

The 92 patients with risk factors also received a maximum prescribed weekly dosage MTX of 12.5 mg/week (range 0–35) with a median cumulative MTX dose of 2256 mg (0–20 235) during a median follow-up period of 219 weeks (range 16–1763 weeks) (this includes baseline biopsies).

Histology

Patients underwent between 1 and 9 (median 2) biopsies per person, and collectively 278 liver biopsies were sampled during the study period. Fifty-six biopsies were performed at baseline. Table 1 lists the distribution of the various Roenigk scores among all liver biopsies taken as well as those taken at baseline. Eighty-eight patients (71%) did not develop histological abnormalities during treatment (Roenigk grade I), 18 patients (14%) had Roenigk grade II, 15 had (12%) grade IIIa, two had (2%) grade IIIB and two (2%) had grade IV. (Table 1.) Characteristics about the patients without histological abnormalities during treatment are shown in Table 2.

A total of 69 patients had two or more liver biopsies. Intra-individual comparison between first and last biopsy demonstrated that the histological findings remained unchanged in 72%, improved in 3% of patients but deteriorated in a further 25%.

Table 1. Risk factors per Roenigk-score

	Roenigk (%)					
	1	2	3a	3b	4	*P**
(a) Liver biopsies						
Baseline† biopsy ($n = 55$)	44 (80)	8 (15)	2 (4)	0	1 (2)	0.11
Follow-up biopsy ($n = 223$)	154 (69)	31 (14)	33 (15)	4 (2)	1(0,4)	
Total biopsies ($n = 278$)	198	39	35	4	2	
(b) Patients						
No risk factors ($n = 34$)	29 (85)	5 (15)	0 (0)	0	0	0.19
Overweight ($n = 38$)‡	24 (63)	10 (26)	2 (5)	1 (3)	1 (3)	0.01
Alcohol use ($n = 62$)	49 (79)	8 (13)	4 (6)	0	1 (2)	0.67
DM ($n = 9$)	6 (67)	1 (11)	0	1 (11)	1 (11)	0.42

MTX, methotrexate; DM, diabetes mellitus.
* Chi-square: Roenigk = 1 vs. Roenigk > 1 (Fisher's exact where appropriate).
† Baseline = cum dose MTX ≤ 600 mg.
‡ Missing values = 20.

Table 2. Characteristics of patients with no histologic abnormalities during treatment

	Patients without histologic injury ($n = 88$) (71%)
Median maximum weekly dosage MTX	12.5 mg (range 0–25)
Median cumulative dosage MTX	2352 mg (range 0–16722)
Median duration follow-up period	214 weeks (range −238–1761)
Risk factors	
Alcohol	37
Alcohol > 14U/week	5
DM	8
Adipositas	25

MTX, methotrexate; DM, diabetes mellitus.

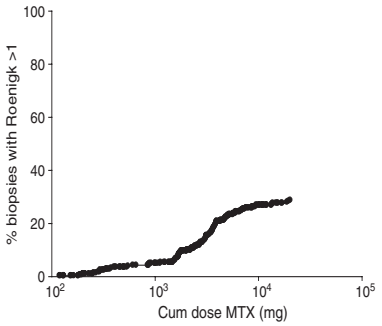
Table 1 also lists the distribution of Roenigk score per risk factor. Those with overweight and/or DM had higher Roenigk scores in comparison to patients without risk factors. In contrast, we did not observe an adverse effect of alcohol use on histological scores.

Association between cumulative dose MTX and liver injury

We observed that histological progression to Roenigk grade 2 or higher, mostly occured when the cumulative MTX dose was between 1500 and 6000 mg. Higher dosages did not lead to increased liver injury as the prevalence of progression to a higher Roenigk score levelled beyond a cumulative MTX dosage of 6000 mg (Figure 1.)

Risk factors, cumulative doses and development of liver damage

Next, we assessed whether biopsies taken from patients with risk factors ($n = 190$) progressed faster to higher Roenigk score in comparison to those biopsies from patients without known risk factors ($n = 88$) (Figure 2.) The presence of obesity, and/or DMD led to progression to a higher Roenigk score (Roenigk grade >1), at earlier cumulative MTX dosages, while alcohol did not.



Cum dose MTX	10^2	10^3	10^4
— Patients at risk (Roenigk = 1)	52	126	20

Figure 1. Percentage of biopsies with Roenigk >1.

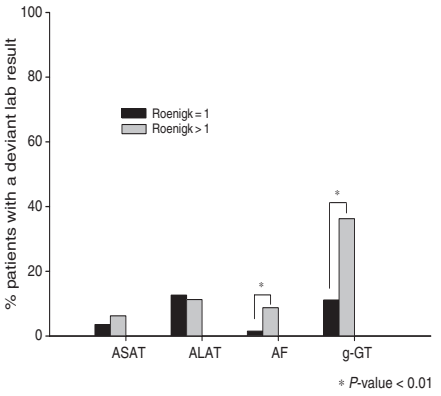
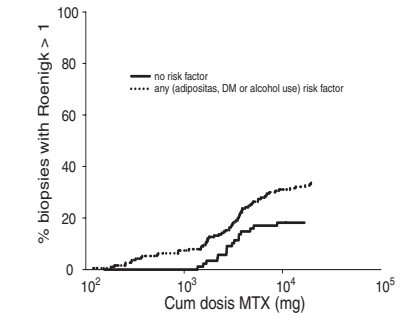


Figure 3. Deviant lab results.



Cum dose MTX	10^2	10^3	10^4
Patients at risk (Roenigk = 1)	15	45	12
--- Patients at risk (Roenigk = 1)	37	81	8

Figure 2. Percentage of biopsies with liver injury (any risk factor vs. none).

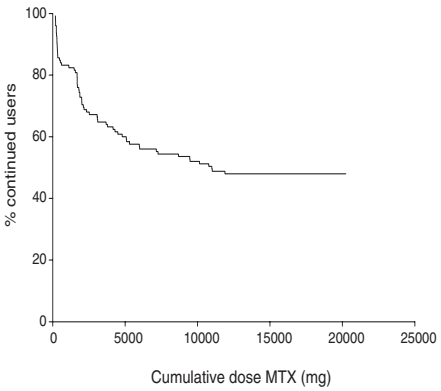


Figure 4. Percentage of continued users.

Liver enzymes and histological changes

Next, we examined the effect of the Roenigk score on the levels of four different liver enzyme tests (γ-GT, AP, ASAT and ALAT). We observed that a higher proportion of patients who had had a liver biopsy with a Roenigk classification ≥2 had γ-GT beyond the upper limit of normal (Odds ratio 1.80; 95% CI 1.30–2.49) (Figure 3.) This contrasted with ASAT and ALAT serum concentrations that did not correlate with Roenigk scores. On the other hand, patients with Roenigk ≥2 had significantly higher AP and ASAT compared with those with Roenigk = 1, but values were well within normal range. All other laboratory tests did not correlate with the histological findings.

Continuation of therapy

Sixty patients (48%) still continued MTX at the end of the studied period. The remaining 52% (65 patients) had stopped using MTX. Most often MTX was stopped relatively early after initiation of MTX, and most patients who had stopped did so at cumulative doses of <5000 mg. (Figure 4). Three patients died during therapy but no deaths were attributed to use of MTX or liver disease related. Reasons mentioned for discontinuation are summarized in Table 3.

Table 3. Reasons for discontinuation methotrexate (MTX) treatment

Reasons to stop MTX therapy	$n = 65$ (%)
Persisting abnormal liver function tests	13 (20)
Dissatisfying results on MTX therapy	9 (14)
Strongly improved skin	9 (14)
Subjective side-effects	7 (11)
Histological abnormalities	6 (9)
Others	21 (32)

Clinical outcome

Biopsies

Pathology results have a limited effect on clinical treatment. Fibrosis/cirrhosis (Roenigk ≥ 3a) was detected in 41 biopsies from 24 patients at some point during MTX treatment. Despite these pathological findings, MTX therapy was continued in 26/41 biopsies. Histological results led to discontinuation in only 6/41 biopsies. In only 9/41 biopsies, treatment was stopped regardless of the results from pathological examination of the liver biopsy. Repeated liver biopsy in Roenigk 3 patients was useful in the clinical treatment.

Patients

In 16 patients, liver biopsy was repeated after finding significant abnormalities at earlier assessments (average of 14 months). Histology did not change in five, while it improved in 10 patients (in five patients to a stage without fibrosis). The liver histology worsened in a single patient.

DISCUSSION

One of our aims was to establish the prevalence of MTX-induced liver injury because the large variation in previous literature.[2, 9, 10] In this substantial cohort of 278 liver biopsies taken over 30 years MTX-induced liver fibrosis (≥Roenigk grade 3a) was only seen in 15% (19 patients) of our population. Progression to a higher stage of liver injury mostly occurred at a cumulative dose-range between 1500 and 6000 mg, which translates to at least 2 years of MTX treatment at 15 mg weekly. Progressive development of liver damage was infrequent above 6000 mg MTX (after about 8 years with 15 mg weekly) when patients have proven to tolerate high cumulative dosages MTX without previously obtaining pathological hepatic differences. In contrast to our data, others found that the cumulative probability of developing advanced hepatic fibrosis doubled after 5000 mg.[9, 10]

We carefully monitored our patients for MTX induced toxicity by regular liver biopsies. As such our practice mirrors the international guidelines and as such the data can be viewed as a field study for implementation of these guidelines.[13, 14] As a consequence, it is unlikely that we missed patients who were treated with MTX and actually developed MTX-hepatotoxicity.

Most long-term observational studies in rheumatoid arthritis indicate that toxicities, rather than lack of efficacy, are the most common cause of discontinuing MTX.[15] This appears to be in contrast with the situation in psoriasis. We and others found that low-dose, long-term MTX treatment seems to be relatively safe and effective, and that undesired side effects lead to discontinuation in a minority.[16]

Another aim of our study was to delineate potential contributing factors to the development of MTX-induced liver injury. Risk factors play an important role in the development of liver fibrosis. At any given cumulative MTX dosage, patients with obesity and or DM had a significantly worse liver histology compared with patients without these risk factors. The presence of fatty infiltration (steatosis) of the liver is highly prevalent among obese and or diabetic patients, and probably plays a role. This reminds us of the situation in non-alcoholic steatohepatitis (NASH) where the presence of steatosis is associated with hepatitis with necroinflammation and pericellular fibrosis.[17] Indeed, a recent case series showed that the presence of NASH in psoriatic patients contributes to the hepatotoxicity of MTX.[18] These data suggest that histological monitoring of MTX toxicity could be tailored to obese patients with or without DM. Other researchers have emphasized the role of alcohol consumption as a risk factor for MTX-induced fibrosis.[10] Surprisingly, we could not confirm this finding in our study and is possible that the association is indeed absent in our cohort. On the other hand, the retrospective nature of our study carries inherent limitations, which may hamper the interpretation of the data on alcohol use. The retrospective nature of our study could have resulted in the under-reporting of risk factors. But even so, we found a significant correlation between the percentage of patients with liver disease and the presence of any risk factors. On the other hand, we cannot rule

out the possibility of entry bias, e.g. that physicians do not consider MTX as a therapeutic option for psoriatic patients with a history of alcohol (ab)use.

We detected a correlation between grades of histological change and γ-GT. In 40% of patients with Roenigk >1, γ-GT was significantly elevated. In contrast, ASAT, ALAT, and AP serum concentrations were not elevated beyond the normal range for any of the Roenigk grades, which fits with other literature data.[10, 19] One of the clinical consequences is that as a whole, normal liver enzymes do not exclude progression of liver injury. Conversely, elevated liver enzymes do not necessarily correlate with MTX induced hepatotoxicity. In this respect, liver enzymes serve as a poor predictor for MTX induced liver injury. One exception may be γ-GT levels, as our data are consistent with the notion that they may serve as a marker for MTX hepatotoxicity. Regular PIIINP measurements might be of additional value in monitoring these patients.[11, 12]

In our study, patients with advanced MTX-induced fibrosis and cirrhosis (Roenigk ≥ 3a) ran a benign course. Liver histology did not deteriorate (and sometimes even improved) under ongoing MTX therapy. Our relatively large series that documented a large number of biopsies over a 20-year period confirms those of two small studies.[9, 10]

In conclusion, MTX-induced fibrosis occurs in a minority of patients. Liver histology deterioration is seen mostly at cumulative MTX dosages between 1500 and 6000 mg, which call for close monitoring at this stage. Our study confirms that normal liver function tests do not guarantee normal liver histology. Furthermore, risk factors play an important role in the development of liver injury and they should be taken into account before the decision is made to start MTX. Obese patients with or without DM should be monitored closely by regular liver biopsies. From our studies it is difficult to indicate a correct interval, but it is probably more often than stated in the guidelines.

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CLINICAL STUDIES

Biochemical and biophysical assessment of MTX-induced liver fibrosis in psoriasis patients: Fibrotest predicts the presence and Fibroscan® predicts the absence of significant liver fibrosis

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Abstract

Background: Methotrexate (MTX) use is associated with hepatic fibrosis in psoriasis patients. To monitor this serial liver biopsies were performed. The Fibroscan® and the Fibrotest are two novel, non-invasive methods that might be able to assess MTX-induced hepatic fibrosis. *Aim:* Evaluating the accuracy and feasibility of the Fibroscan® and Fibrotest to detect *significant* MTX-induced liver fibrosis in psoriasis patients. *Methods:* We assessed 24 psoriasis patients who had a recent liver biopsy during MTX use. The results from the Fibroscan® and Fibrotest were compared with liver histology. *Results:* Fibroscan® values ($n = 20$) ranged between 3.3 and 18.4 kPa (median value 6.4 kPa) and correctly identified 88% of the patients without significant liver fibrosis (Metavir score $<$ F2, Fibroscan® $\leq$ 7.1 kPa). The Fibrotest identified 83% of the patients *with* significant liver fibrosis (Metavir score $\geq$F2, Fibrotest >0.31). *Conclusion:* In this population, Fibrotest accurately predicted the presence of significant liver fibrosis while the Fibroscan® accurately predicted the *absence* of significant liver fibrosis in MTX users. This suggests that a combination of Fibrotest and Fibroscan® should prospectively be evaluated in monitoring and detecting significant MTX-induced liver fibrosis in psoriasis patients.

Methotrexate (MTX) is the most commonly prescribed systemic drug for severe psoriasis. There is substantial evidence to suggest that it acts by inhibiting DNA synthesis. Probably as a consequence, it possesses potent anti-inflammatory effects on T-cell mediated immune responses as it inhibits proliferation or induces apoptosis in activated T-cells and blocks abnormal rapid epidermal cell proliferation, both responsible for the characteristic skin lesions in psoriasis (1). Low-dose treatment with MTX is regarded as an effective therapy for psoriasis.

However, one of the dreaded long-term side effects includes liver fibrosis and cirrhosis because of MTX hepatotoxicity. Therefore, frequent evaluation of liver enzymes and periodic liver biopsy are recommended during therapy. Specifically, dermatologic guidelines call for a liver biopsy at every 1500 mg cumulative dose (2). Although liver biopsy is considered as the golden standard for the assessment of histological changes (3), complications such as postprocedural pain and bleeding limit its clinical use, with a 0.01–0.1% risk of mortality (4).

Therefore, there is a pressing need for alternative, non-invasive and reliable methods to monitor MTX-induced liver injury in psoriasis patients. Non-invasive tools like blood tests and Fibroscan® are being used in patients with chronic liver diseases (5). This study evaluates two non-invasive methods for detection of significant liver fibrosis in MTX-treated psoriasis patients.

The first test is a recently developed patented artificial intelligence algorithm (Fibrotest) that has been validated to detect fibrosis in hepatitis C patients (6). It is a biochemical fibrosis index that needs input values of five serum markers and is corrected for age and sex, leading to a composite value (between 0 and 1) to determine the presence of significant liver fibrosis (7, 8).

As a second test, we measured liver elasticity, using one-dimensional transient elastography, the Fibroscan®. As liver stiffness roughly correlates with the degree of hepatic fibrosis, it can serve as a non-invasive test for fibrosis. This latter approach seems promising for the assessment of fibrosis and cirrhosis in patients with hepatitis C and a fine correlation has been established between significant fibrosis (F2 according to the Metavir histology grade) and elastography (Fibroscan®) outcome (7–10).

We compared the test characteristics of Fibrotest and Fibroscan® in a cohort of psoriasis patients treated with MTX and compared the results with the histology of the liver. Our ultimate aim was to identify effective test alternatives for a liver biopsy.

Patients and methods

Patients

Our study population was drawn from 60 psoriasis patients who were on MTX treatment at the end of 2005. Patients who underwent a percutaneous liver biopsy within 18 months of Fibrotest (Biopredictive, Paris, France) and Fibroscan® (Echosens, Paris, France), as part of their regular MTX monitoring procedure, were eligible for inclusion in this study. We contacted 34 patients and asked them to participate in the study. Twenty-four patients agreed to participate and gave their written informed consent. We collected information about the risk factors for liver damage such as excessive alcohol consumption, obesity, diabetes mellitus and chronic viral hepatitis using a structured interview.

Histological assessment

Liver biopsies for histological evaluation of MTX effects were obtained using a 1.6 mm diameter Menghini-type needle via the right intercostal approach with local lidocaine anaesthesia (Hepafix, Braun, Melsungen, Germany). The biopsy site was marked by ultrasound examination. There were no complications. Biopsy specimens were fixed in 4% formaldehyde and subsequently embedded in paraffin. Haematoxylin and eosin-stained sections of liver tissue were examined for steatosis, lobular and portal tract inflammation, hepatocyte necrosis and nuclear variability. A von Gieson stain for collagen was assessed for the presence of pericellular and perivenular fibrosis, as well as the expansion of the portal tracts. All biopsies were revised and analysed by two independent investigators (M. A. M. B and J. S.) and verified by an experienced pathologist (J. H. v. K.). Disagreements were resolved by consensus. All liver biopsy specimens were scored according to the Metavir histology score (11). Furthermore, the number of portal tracts were noted and all liver biopsies (previously taken liver biopsies included), as well as their results (Metavir score) were reported. A consistent result was defined as a Roenigk score that was stable over time or increased gradually. An inconsistent result was defined as a decrease of more than two grades or a random pattern with an increase and later a decrease with one grade on Roenigk classification.

Non-invasive measurements of fibrosis

Fibrotest

Blood samples were collected to analyse five serum markers included in the Fibrotest score. For this purpose, we measured the following biological parameters: γ-glutamyl-transpeptidase (γ-GT), total bilirubin level, α2-macroglobulin, apolipoprotein A1 and haptoglobin. In addition, we measured albumine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase and bilirubine.

Measurements were performed immediately on fresh obtained samples using validated methods. The results were used as input for the Fibrotest. This is a patented artificial intelligence algorithm that generates a measure of liver fibrosis. It provides a numeric quantitative estimate of liver fibrosis ranging from 0.00 to 1.00. It is a continuous linear biochemical assessment of fibrosis stage, which corresponds with the stages F0–F4 of the Metavir scoring system. Based on the literature data, we chose a cut-off value of 0.31 to identify patients with significant fibrosis ($\geq$F2) (8, 12, 13). Fibrotest results were kindly provided by T. Poynard, University Paris VI, Paris, France (www.biopredictive.com).

Fibroscan®

We performed the Fibroscan® measurements on the same day as the measurements of the biological parameters that are used for the Fibrotest. The Fibroscan® is an ultrasound transducer that generates vibrations that cause a slow elastic shear wave. The propagation and velocity of the wave in the liver are tracked by pulse-echo ultrasound and correlate to tissue stiffness. Measurements were performed on the right lobe of the liver, at the same target area for liver biopsy. The procedure was performed through the intercostal space while the patients were lying on their backs with their arms in maximal abduction behind their heads.

Each patient underwent a series of 10 validated electrographic measures. The success rate was calculated as the number of validated measurements divided by the total number of measurements. The results were expressed in kilopascals (kPa) and according to Castera a cut-off value of 7.1 kPa was chosen to identify patients with significant fibrosis ($\geq$F2) (7). The median value was considered to be representative for the elasticity of the liver. Only procedures with 10 validated measurements and a success rate of at least 60% were considered to be reliable. Fibroscan® assessments were performed by an experienced physician (R. J. d. K.) who was strictly blinded to the histological outcome.

Cumulative MTX dose and the three tests

We next went on to test the effect of cumulative MTX usage on the presence of liver fibrosis as assessed by the three tests under study. The presence of liver fibrosis was defined as the presence of Metavir $\geq$F2 on a liver biopsy specimen.

Statistical analysis

Frequency tables were provided for both demographic and clinical information (cumulative MTX dose, alcohol consumption and the presence of diabetes mellitus). The diagnostic performance of the non-invasive methods for liver fibrosis was measured as sensitivity, specificity, positive predictive value, negative predictive value and accuracy for the presence and absence of significant fibrosis (>F2). The results of both tests were drawn in a scatterplot with regression lines of individual Metavir scores in relationship with the results of Fibrotest and Fibroscan®. Furthermore, we compared the two non-invasive tests using the non-parametric Spearman correlation test, both continuously and subdivided for Metavir score. Finally, the relation between the cumulative MTX dose and the results of the three tests was tested with the Wilcoxon's rank sum analysis. All statistical analyses were undertaken with SAS statistical software (SAS institute, Minneapolis, MN, USA), version 8.2.

Results

Demographics

We included 24 psoriasis patients (13 females, 11 males) with a mean age of 55 years (range 34–73). The reasons for performing a liver biopsy were as follows: all patients underwent a liver biopsy in keeping with the guideline after a median dosage of 1635 mg MTX (range 162–2354 mg). Only one patient had elevated liver enzymes more than twice the upper limit. Sixteen patients were biopsied before.

Table 1. Main demographic and clinical features of the studied population

	N = 24
Male gender (%)	11 (46)
Median age, years (range)	55 (34–73)
Median body mass index, kg/m^2 (range)	26 (20–38)
Diabetes mellitus (%)	4 (17)
Median cum dose MTX, mg (range)	3352 (314–20 235)
Median Fibroscan, kPa (range)	6.4 (3.3–18.4)
Median Fibrotest (range)	0.32 (0.06–0.93)

MTX, methotrexate.

Table 2. Three patients with inconsistent histology

Patients	Histology according to Roenigk score				
1	R1	R3a	R1	R3a	R2
2	R1	R3a	R1	R1	
3	R1	R1	R3a	R2	R1

The median body mass index was 26 kg/m^2 (range 20–38 kg/m^2) and 14 patients were considered to be overweight (defined as body mass index >25 kg/m^2). Ten patients consumed alcohol, while a single patient was an excessive consumer (>14 U/week) and four patients had diabetes mellitus. Patients received a median cumulative MTX dose of 3352 mg (314–20 235) during a median follow-up period of 346 weeks (111–2162). Four patients had a cumulative dose of more than 5000 g. Table 1 shows the main demographic and clinical features of the population studied.

Histology

In the population studied, the median biopsy length was 30 mm (range 10–60 mm). Five patients (21%) had a liver biopsy specimen shorter than 15 mm. The mean number of portal tracts was 22 (SD 9). Scoring with the Metavir classification resulted in five patients with a liver biopsy classified as F0, 13 as F1, 4 as F2, 1 as F3 and 1 as F4.

Sixteen patients had more than one liver biopsy. Thirteen patients (81%) had consistent pathological scoring of their liver biopsies over the years. In only three patients the histology results varied between biopsies (Table 2).

In patient 3, the time period between the first two biopsies was 6 months. Treatment with MTX was discontinued then, but was restarted 14 years later. Histology of the follow-up biopsy 18 months later

Table 3. Diagnostic value of fibrotest and fibroscan in detecting patients with clinically significant fibrosis (METAVIR fibrosis score F2 or greater)

	Fibrotest ≥F2	Fibroscan ≥F2
Optimal cut-off	0.31	7.1 kPa
Sensitivity (%)	83	50
Specificity (%)	61	88
Accuracy (%)	67	70
Positive predictive value (%)	42	33
Negative predictive value (%)	92	86

resulted in an R1. In patient 2, the time period between the second and third biopsy was half a year. The last two biopsies were consistent and the time period between them was 3 years. In patient 1, time periods between biopsies were 3 years, 8 months, 4.5 years and 10 months. The median length of the consistent biopsies was 25 mm (8–85 mm) vs a median length of 24.5 mm (9–45 mm) of the inconsistent biopsies.

Fibrotest

Fibrotest values ($n = 24$) ranged between 0.06 and 0.93 (median value 0.32). Table 3 shows the diagnostic test properties for the Fibrotest with respect to detection of significant liver fibrosis (Metavir fibrosis score ≥F2). Figure 1 shows the agreement in Metavir score between the fibrotest and histology. The results show that Fibrotest correctly identifies those patients with significant fibrosis in 83% of the cases, indicating good screening properties (Metavir fibrosis score ≥F2, Fibrotest < 0.31).

Fibroscan®

The mean number of measurements per patient was 12.1 ± 2 (range: 10–18) to obtain 10 evaluable values. The success rate of liver elastography measurements was 85 ± 12%. The total procedure failed in four (17%) patients because of the presence of obesity. These patients had a body mass index (BMI) of 35.4, 32.4, 38.2 and 32.7 kg/m^2, Metavir histology scores F0, F1, F3 and F2 and Metavir Fibrotest scores F0, F3, F3 and F4.

Liver stiffness measurements ($n = 20$) ranged between 3.3 and 18.4 kPa (median value 6.4 kPa). Table 3 shows the diagnostic test properties for the Fibroscan® in our study population with respect to detection of significant liver fibrosis (Metavir fibrosis score ≥F2). The test detected 88% of the patients without significant fibrosis (Metavir fibrosis score < F2) in this population, indicating good diagnostic properties. Figure 1 shows the agreement in Metavir score between the fibrotest fibroscan and histology.

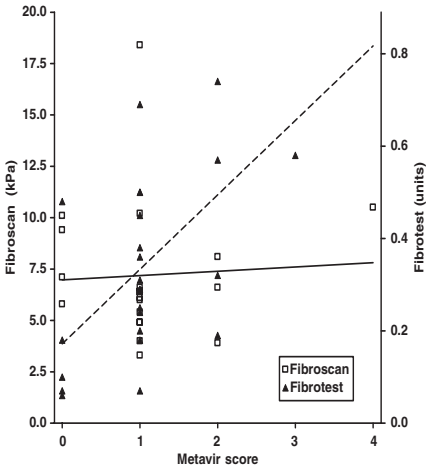

Fig. 1. Scatterplot representing all observations and regression lines for both Fibrotest (dashed line) and Fibroscan® (regular line).

Table 4. Relationship cum MTX dose vs test

	Median cum dose MTX, mg (range)		
	Metavir < F2	Metavir ≥F2	*P**
Histology	3879 (314–20 235)	2791 (1445–3882)	0.45
Fibroscan	4396 (314–18 502)	4467 (1400–15 173)	0.40
Fibrotest	2166 (314–6000)	4026 (1445–20 235)	0.10

*Tested with the Wilcoxon's rank-sum test.
MTX, methotrexate.

Relationship cumulative MTX dose results of three tests

There was no effect of cumulative MTX dosing on the presence of liver fibrosis. Patients with significant liver fibrosis (Metavir fibrosis score ≥F2) did not have a higher cumulative MTX dose than patients without significant liver fibrosis as assessed by any of the three tests (Table 4).

Discordance between fibrotest and fibroscan®

In nine patients, Fibroscan® and Fibrotest resulted in different Metavir scores with a discordance of two stages. In four of them, the total Fibroscan® procedure failed because of the presence of obesity. In the remaining five, biopsy length was significantly shorter

compared with the biopsy length of the remaining patients. There was no significant difference in BMI between those patients. Other potential confounders for failure of Fibrotest like Gilbert, haemolysis and acute inflammation were ruled out.

Discussion

Our aim was to evaluate the accuracy and feasibility of two non-invasive methods, Fibrotest and Fibroscan®, in the detection of significant MTX-induced liver fibrosis, using liver biopsy as the gold standard, in a cohort of psoriasis patients. Specifically, in our population we found that the Fibroscan® is a good diagnostic test for *excluding* significant liver fibrosis ($\geq$F2 on the Metavir score) but less equipped in *detecting* significant fibrosis ($\geq$F2) in this population. On the other hand, we found that the fibrotest is a good screening test in detecting significant fibrosis. This suggests that a complementary use of both Fibrotest and Fibroscan® is beneficial in establishing the grade of liver fibrosis in MTX-induced liver fibrosis in psoriasis patients and might be instrumental in reducing the need for liver biopsies.

The Fibroscan® results of this study accord with those collected from the literature. It had a comparable accuracy in excluding significant liver fibrosis in a sample of patients with hepatitis C, but also in a series of patients with Crohn's disease on MTX treatment (7, 8, 10, 14, 15).

Our sample contained only a few patients with F3 or F4 liver fibrosis. Thus, we mainly based our conclusion on patients with a Metavir histology grade F0–F2. However, in another study fibroscan® was prospectively investigated in a large cohort of patients with chronic liver disease of various aetiologies including 144 (20%) of patients with F3/F4 fibrosis. There diagnostic performances for $\geq$F2 were comparable to our results, sensitivity 50% vs 64% and specificity 85% vs 88% (9).

Because continuous long-term use of MTX is associated with hepatotoxicity, frequent evaluation of liver function tests and periodic liver biopsy are recommended during therapy. Specifically, dermatologic guidelines call for a liver biopsy at every 1500 mg cumulative dose (2). Conventional liver enzyme tests (ALT or g-GT) correlate poorly with histological changes (3). Therefore, liver biopsy is considered as the gold standard method in the assessment of histological changes. A recent study by our group about liver injury in long-term MTX treatment in 125 patients with psoriasis with 278 liver biopsies found that progression to a higher stage of liver injury mostly occurred at a cumulative dose range between 1500 and 6000 mg, which translates to at least 2 years of MTX treatment at 15 mg weekly. Furthermore, in this study, ASAT, ALAT and AF serum concentrations were not elevated beyond the normal range for any of the Roenigk grades (16).

A liver biopsy has some important limitations. It is an invasive procedure and carries several known risk factors such as pain, localized bleeding and less often pneumothorax, haemothorax, bile peritonitis, haemobilia, and inadvertent puncture of the kidney or intestine (17, 18). The complication risk of a liver biopsy is approximately 1–2%, with a 0.01–0.1% risk of mortality (4). Furthermore, it causes anxiety, which is an issue, as most psoriasis patients on the MTX require repeated liver biopsy during the course of their treatment. Another limitation of the liver biopsy is the possible sampling error, an intra- and inter- pathologist inconsistency in observations and that there are discontinuous and hence semi-quantitative histological scoring systems (2, 3, 7, 12, 15, 17, 19, 20). In three patients, histology results varied considerably between biopsies (inconsistent). In one patient, this might have been caused by the improvement of liver tissue during a long period in which the patient had not been treated with MTX, which favours the hypothesis that MTX-induced injury is reversible and in another patient the last two biopsies were consistent. To conclude, only in one patient histology results might have reflected a sampling error of liver biopsy.

There is an urgent need for alternative, non-invasive and reliable methods of monitoring and detecting MTX-induced liver injury in psoriasis patients. Fibrotest has been studied in patients with hepatitis B and C, alcoholic liver disease and in patients with non-alcoholic fatty liver disease (21–27). Previous studies found that non-invasive assessment of liver stiffness by use of the Fibroscan® is a reliable test to detect significant fibrosis or cirrhosis (8–10, 14, 15). The positive predictive value for detecting significant fibrosis is only 76%, but the combined use of Fibroscan® and Fibrotest does allow the evaluation of hepatitis C liver fibrosis (7, 28). In one study the stepwise combination of non-invasive markers of liver fibrosis improved the diagnostic performance in chronic hepatitis C patients. The need for liver biopsy in that study was reduced by 50–70% (28).

The main advantage of Fibroscan® compared with the Fibrotest is that it provides a direct quantative physical parameter. In obese patients, the fatty thoracic belt attenuates both elastic waves and ultrasound, rendering it difficult or even impossible (15). In our study, 12 patients suffered from obesity and in three of

them the Fibroscan® failed. Psoriasis patients tend to be more obese compared with the normal population, with a prevalence of 34% in psoriasis patients vs 18% in the normal population (29), which might make this approach less successful in detecting MTX-induced liver injury in psoriatic patients.

In the search for a non-invasive method to monitor and detect MTX–induced liver injury, Amino terminal peptide of type III procollagen (PIIINP) was studied before (4, 30). PIIINP may serve as an alternative for routinely performed liver biopsies when measured levels at regular intervals are consistently normal in otherwise healthy adults. This is because PIIINP is not an organ-specific protein and may be raised in children and various pathological states associated with development of fibrosis including inflammatory arthritis, scleroderma, hyperthyroidism, scar formation following burns and myocardial infarction (4, 30). The advantage that Fibrotest offers over PIIINP is that one cross-sectional measurement is sufficient to judge whether liver fibrosis is present.

A limitation of our study is the relatively small study population, which may limit the precision of the effect estimates. Furthermore, as mentioned above, a liver biopsy, although considered as the gold standard, can have sampling variability problems. This might have been the case in our study because some patients had small liver biopsies. However, in our study liver biopsy length compares favourably with that of some other studies (9, 31) and only one patient had a liver biopsy with less than 10 portal tracts.

The pattern of results indeed supports the notion that the diagnostic test properties of Fibroscan® and screening properties of Fibrotest should be evaluated in a prospective manner. Specifically, we would like to propose a prospective controlled trial that evaluates the combined use of non-invasive markers such as the Fibrotest, Fibrotest and PIIINP in the detection of MTX-associated fibrosis (7, 28).

Although it should be kept in mind that this is a small pilot study, based on our results we conclude that the Fibrotest seems to be good in *detecting and* the Fibroscan® seems to be good in *excluding* significant MTX-induced liver fibrosis ($F \geq 2$) in patients with psoriasis treated with MTX. This suggests that the combined use of Fibrotest and Fibroscan® may be beneficial in establishing the grade of liver fibrosis in MTX-induced liver fibrosis in psoriasis patients.

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STUDY

Reliability of the Roenigk Classification of Liver Damage After Methotrexate Treatment for Psoriasis

A Clinicopathologic Study of 160 Liver Biopsy Specimens

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Objective: To determine the interobserver reliability of the Roenigk score as a classification system of liver damage and its possible consequences for clinical practice.

Design: Retrospective study.

Setting: Academic research.

Patients: One hundred sixty liver biopsy specimens from patients with psoriasis receiving methotrexate treatment were rereviewed and analyzed blindly by an experienced pathologist with an interest in liver pathologic conditions.

Main Outcome Measure: Interobserver variation was evaluated using κ statistics.

Results: A high concordance was present in the evaluation of the Roenigk grade of fibrosis (weighted κ=0.73; 95% confidence interval, 0.63-0.83). Agreement was good regarding the number of biopsy specimens for patients whose clinical management should be changed (κ=0.71; 95% confidence interval, 0.56-0.87).

Conclusion: The Roenigk classification in the assessment of liver fibrosis is a reliable scoring system.

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HEPATIC FIBROSIS AND CIRrhosis represent a consequence of methotrexate treatment in patients with psoriasis.[1-6] Therefore, the assessment of liver damage is essential in the clinical management of these patients. Sequential liver biopsies followed by Roenigk grading by a pathologist are the mainstay in the assessment of the stage and degree of liver damage.[7-9] Unfortunately, liver biopsies may be associated with sampling error, potential complications, and interobserver variability.[1,7,9-13]

Methotrexate-associated liver damage in patients with psoriasis is graded according to the Roenigk classification.[1] The results of the Roenigk scoring system should be reproducible, with little interobserver error.

The Roenigk classification was developed by the Psoriasis Task Force (led by dermatologists), is based on clinical observations, and has been recommended in the American Academy of Dermatology guidelines for monitoring methotrexate-induced liver injury.[1,9,14] However, the Roenigk grading system is subjective, including some features (such as nuclear pleomorphism) of unclear significance, and is insensitive to small changes, particularly when assessing fibrosis.[1,15,16] Although scoring seems to consider changes such as steatosis and inflammation, their presence or absence has no weight in the allocation to more advanced grades. The system categorizes all biopsy specimens with more than minimal fibrosis as advanced fibrosis (Roenigk grade 3b) and overestimates the degree of histologic change. Accurate assessment is essential because misclassification of pathologic changes affects clinical management. For example, if the degree of fibrosis is upgraded from none (Roenigk grade 2) to mild (Roenigk grade 3a), guidelines call for a second liver biopsy within 6 months instead of a 1.5-g cumulative dose of methotrexate.[9] In the case of Roenigk grade 3b or 4, the guidelines recommend discontinuation of therapy.

In some European countries, the number of liver biopsies has declined for several reasons. The use of the aminoterminal propeptide of type III procollagen (PIIINP) has reduced the number of liver biopsies in Scandinavia and in Great Britain. Until recently, no noninvasive method

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Table 1. Roenigk Classification System

Fatty Change	Nuclear Pleomorphism	Fibrosis	Necroinflammation	Roenigk Grade[a]
Mild or none	Mild or none	None	With or without mild portal inflammation	1
Moderate or severe	Moderate or severe	None	Moderate or severe portal inflammation	2
With or without	With or without	Mild (fibrosis extending into acini)	With or without	3a
With or without	With or without	Moderate or severe	With or without	3b
With or without	With or without	Cirrhosis	With or without	4

[a]See the "Pathologic Examination" subsection of the "Methods" section for an explanation of Roenigk grades.

has been available that could completely replace the liver biopsy. In the case of a persistently elevated PIIINP, liver biopsy is still advised.[11,13,17] Given the critical nature of this tool, we evaluated interobserver variation using a sample of 160 liver biopsy specimens from methotrexate-treated patients with psoriasis.

METHODS

PATIENTS

We evaluated interobserver variation between several different pathologists with an interest in liver pathologic routine clinical practice and 1 of us (J.H.v.K.) in the assessment of the histopathologic degree of liver damage according to the Roenigk scale in patients with psoriasis receiving methotrexate treatment. All pathologists were trained at the same department of pathology at the same hospital.

One hundred twenty-five patients with psoriasis had undergone 278 liver biopsies while receiving methotrexate treatment from November 1, 1976, to December 31, 2005. We excluded biopsies performed before December 31, 1995, because these specimens were unavailable for review. In addition, 9 biopsy specimens were excluded from analysis (6 because they were unavailable from the department's archives, 1 was too small to evaluate the degree of fibrosis, and 2 because the van Gieson–stained slide was unavailable). One hundred sixty liver biopsy specimens from 95 patients were reexamined independently by 1 of us (J.H.v.K.) who was blinded to the clinical details of the patients. Liver biopsy specimens were graded according to the Roenigk classification (**Table 1**).[1]

HISTOLOGIC EXAMINATION

Percutaneous liver biopsy was performed via a right intercostal approach using local lidocaine hydrochloride anesthesia. The biopsy specimen was immersed in 2% formaldehyde and was subsequently fixed with paraffin. Hematoxylin-eosin–stained sections of liver tissue were examined for steatosis, nuclear variability, hepatocyte necrosis, and lobular and portal tract inflammation. A van Gieson stain for collagen was used to assess for the expansion of the portal tracts and for the presence of pericellular and perivenular fibrosis.

PATHOLOGIC EXAMINATION

All liver biopsy specimens, sampled as part of the monitoring process of methotrexate-induced hepatic injury, were graded according to the Roenigk classification. A description of the Roenigk classification is given in Table 1. Roenigk grade 1 indicates normal tissue with no fibrosis, no or mild portal inflammation, and no or mild fatty changes and nuclear pleomorphism. Grade 2 indicates no fibrosis and moderate or severe fatty changes, nuclear pleomorphism, and portal inflammation. Grade 3a indicates mild fibrosis, portal fibrotic septa, extension into the lobuli, and portal tract enlargement. Grade 3b indicates moderate or severe fibrosis. Grade 4 indicates cirrhosis, regenerating noduli, and bridging of the portal tracts.

STATISTICAL ANALYSIS

To judge the degree of interobserver agreement, we calculated weighted κ statistics for the 5-point Roenigk scale. For analysis of clinical consequences, we dichotomized the Roenigk score into "no changes of treatment necessary" (Roenigk grades 1 and 2) and "change of treatment necessary" (Roenigk grades 3a, 3b, and 4) for all observations. For agreement of the dichotomized data, we again used κ statistics. Interpretation of the κ statistics was performed using the scale described by Landis and Koch,[18] in which κ statistics less than 0.4 indicate poor agreement, κ statistics between 0.4 and 0.6 indicate moderate agreement, between 0.6 and 0.8 good agreement, and greater than 0.8 indicate excellent agreement.[19]

To visualize agreement, we plotted a Bland-Altman curve for the 5-point Roenigk score. Using a 2-sided *t* test, we tested whether the overall mean differences differed statistically significantly from 0. All analyses were undertaken using statistical software (SAS version 8.2; SAS Institute Inc, Cary, North Carolina).

RESULTS

DEMOGRAPHICS

Ninety-five patients with psoriasis (44 female and 51 male) underwent a liver biopsy between December 31, 1995, and December 31, 2005. The maximum prescribed weekly dosage of methotrexate was 12.5 mg (range, 7.5-25 mg). Patients received a median cumulative methotrexate dose of 2051 mg (range, 119-20 235 mg) during a median follow-up period of 202 weeks (range, 20-1763 weeks).

LIVER BIOPSY SPECIMENS

The concordance between the Roenigk grades as scored during routine assessment and at subsequent scoring by the second pathologist was high (weighted $\kappa = 0.73$; 95% confidence interval, 0.63-0.83). The agreement was higher for biopsy specimens that were graded as Roenigk grade 1, which was the most common Roenigk score (**Figure**). The mean difference was 0.03 and did not significantly differ from 0 ($P > .05$). Among liver biopsy specimens that resulted in a change of clinical management (Roenigk grades 3a, 3b, and 4), we like-

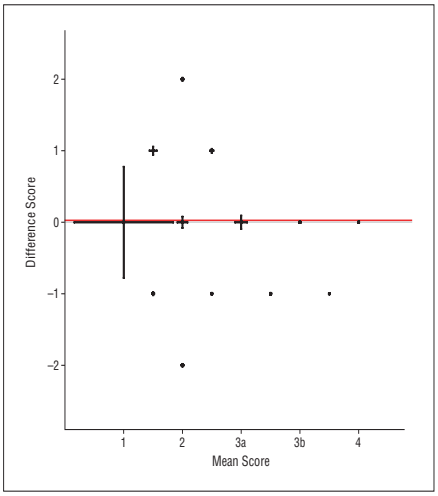

Figure. Bland-Altman curve. The graph shows the absolute difference between the initial and subsequent scores (on the y-axis) against the mean of both scores (on the x-axis) for each observation. In the graph, the size of the crosses indicates how often a pair of observations is found. The red line indicates the 0.03 overall mean difference between the initial and subsequent scores.

wise observed a good correlation (κ=0.71; 95% confidence interval, 0.56-0.87).

The initial routine examination had graded 113 liver biopsy specimens as Roenigk grade 1, 21 as grade 2, 22 as grade 3a, 3 as grade 3b, and 1 as grade 4 (**Table 2**). After reexamination of all liver biopsy specimens by the second pathologist, 118 were graded as grade 1, 18 as grade 2, 18 as grade 3a, 4 as grade 3b, and 2 as grade 4.

Six liver biopsy specimens originally scored as Roenigk grade 1 were scored differently by the second pathologist (3 as grade 2 and 3 as grade 3a). Ten liver biopsy specimens originally scored as grade 2 were subsequently scored differently (2 were upgraded to grade 3a, while 8 were downgraded to grade 1). Nine liver biopsy specimens originally scored as grade 3a were scored differently by the second pathologist (2 as grade 3b, 4 as grade 2, and 3 as grade 1). Finally, 1 liver biopsy specimen originally scored as grade 3b was subsequently upstaged to grade 4.

CLINICAL CONSEQUENCES OF DIFFERENCES IN SCORING

Fourteen biopsy specimens were downgraded or upgraded to such an extent that it would have affected clinical management (**Table 3**). Seven biopsy specimens graded as Roenigk grade 3a were downgraded by the second pathologist to grade 2 or 1. Because of the original grade, follow-up biopsies in 3 patients were performed after 5 to 10 months, and methotrexate treatment in 1 patient was discontinued after 5 months. Three biopsy specimens were upgraded by the second pathologist from grade 1 to 3a, 2 biopsy specimens from grade 2 to 3a, and 2 biopsy specimens from grade 3a to 3b. In the latter 2 cases, this assessment resulted in follow-up biopsies after 9 and 14 months that demonstrated histologic findings corresponding to grade 3a. One of these patients is still being treated with methotrexate. In the other patient, methotrexate treatment was continued, and 2 more biopsies were performed. Both biopsy specimens demonstrated histologic findings corresponding to grade 3a. Methotrexate treatment was discontinued for an unknown reason.

Table 2. Numbers of Slides Assigned to the Roenigk Grades by the Pathologists[a]

Roenigk Grade	First Pathologist	Second Pathologist
1	113	118
2	21	18
3a	22	18
3b	3	4
4	1	2

[a] See the "Pathologic Examination" subsection of the "Methods" section for an explanation of Roenigk grades.

COMMENT

Our objectives were to determine the interobserver reliability of the Roenigk score as a classification system of methotrexate-induced liver damage and to assess the consequences for clinical practice. The results of this study show that the Roenigk classification is a reliable scoring system for the assessment of liver fibrosis.

The study revealed high concordance between the first and second observations. Also, there was good agreement on biopsy specimens that resulted in a Roenigk grade that necessitated change of clinical management (biopsy specimens with grades 3a, 3b, and 4). Although only a small percentage of the biopsy specimens was scored differently by the second pathologist, it would have resulted in a clear change in the clinical decisions made. Grade 3a requires more frequently performed liver biopsies (within 6 months instead of a 1.5-g cumulative dose of methotrexate), and grades 3b and 4 necessitate interruption and cessation of methotrexate treatment.[9]

Periodic liver biopsies are recommended by international guidelines[4,9,14] on methotrexate treatment in patients with psoriasis, and the Roenigk score has been recommended in the American Academy of Dermatology guidelines[4,9,14] to classify methotrexate-induced liver damage. However, the Roenigk scale has not been validated or used (to our knowledge) in the evaluation of any other liver disease.[1] Furthermore, the Roenigk scale is subjective and is insensitive to small changes, particularly when assessing fibrosis.[1,16] Scoring systems for liver damage such as the Metavir, Scheuer, and Ishak classifications are well established for hepatitis C and for some forms of nonviral hepatitis; these scoring systems are more sensitive to small changes, and studies[1,20-24] demonstrated good agreement for fibrosis assessment. As far as we know, there are no studies evaluating the validity and interobserver reliability of the Roenigk score. However, 2 studies compare the Roenigk classification with other scoring systems. One study[15] compares the Roenigk score with a

Table 3. Slides Scored Differently by the Pathologists

Roenigk Grade[a]		No. of Slides Scored Differently	Upgrade or Downgrade	Clinical Consequences
First Pathologist	Second Pathologist			
1	2	3	Upgrade	No
1	3a	3	Upgrade	Yes
2	1	8	Downgrade	No
2	3a	2	Upgrade	Yes
3a	1	3	Downgrade	Yes
3a	2	4	Downgrade	Yes
3a	3b	2	Upgrade	Yes
3b	4	1	Upgrade	No

[a] See the "Pathologic Examination" subsection of the "Methods" section for an explanation of Roenigk grades.

semiquantitative histologic scoring system for the evaluation of hepatic fibrosis in patients with rheumatoid arthritis treated with methotrexate. A statistically significant correlation was found between the 2 classification systems, but the semiquantitative histologic scoring system was much more sensitive than the Roenigk score for the assessment of hepatic fibrosis. Another study[1] compared 3 scoring systems for the evaluation of hepatic fibrosis in patients with psoriasis treated with methotrexate. The Roenigk classification was compared with the Scheuer and Ishak scoring systems and seemed to correlate poorly with both systems.

The already described simplification of the Roenigk classification may have improved the interobserver reliability in our study. This raises the question of whether the Roenigk classification is the best-designed scoring system to classify methotrexate-induced liver injury. However, that was not the objective of our study. The Roenigk classification is used by many pathologists to classify methotrexate-induced liver fibrosis. In this study, it is shown that the interobserver reliability is good.

In 8% of the liver biopsy specimens, a different clinical decision would have been made based on disagreement between the first and second observers. When this leads to more frequently performed liver biopsies, serious consequences arise for the patient. Patients will be at greater risk for morbidity and mortality associated with liver biopsies such as postprocedural pain, bleeding, and (less often) pneumothorax. Also, an increase in liver biopsies has socioeconomic consequences such as absence from work. Unnecessary liver biopsies should be avoided, and there is a need for alternative noninvasive and reliable methods to monitor methotrexate-induced liver injury in patients with psoriasis. Several noninvasive methods have been tested as a screening for liver fibrosis and liver cirrhosis (eg, the Fibrotest, Fibroscan, and PIIINP).[11,13,17,25] Another serious consequence would be the risk of missed pathologic findings that would necessitate discontinuing methotrexate treatment or undergoing another liver biopsy in 6 months.

This study was composed of a rereview of 160 liver biopsy specimens by 1 of us (J.H.v.K.). However, the retrospective nature of the study has some limitations, which might be reflected in the differences in the results found. Slides could have lost some of their stains, and observation of slides serially (by the second pathologist) or individually (by the first pathologist) could have resulted in some of the differences.

One biopsy specimen was excluded from the study because it was too small to evaluate the degree of fibrosis. A hepatologist experienced in performing liver biopsies and in repeating liver biopsy procedures is essential for obtaining adequate specimens and for the safety of the patient.

Based on this study, we conclude that the interobserver reliability of the Roenigk classification is good and that it can be used as a scoring system for methotrexate-induced liver damage. However, the clinical consequences of rereview were substantial. Experienced pathologists with an interest in liver pathologic conditions are recommended, as well as particular attention to biopsy specimens with Roenigk grades 3a and 3b. The search for noninvasive alternatives to liver biopsy should be continued.

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Author Contributions: Dr Berends had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Berends, van Oijen, van de Kerkhof, Drenth, van Krieken, and de Jong. *Acquisition of data:* Berends, Snoek, Drenth, van Krieken, and de Jong. *Analysis and interpretation of data:* Berends, van Oijen, van de Kerkhof, Drenth, van Krieken, and de Jong. *Drafting of the manuscript:* Berends, Snoek, Drenth, van Krieken, and de Jong. *Critical revision of the manuscript for important intellectual content:* Berends, van Oijen, van de Kerkhof, Drenth, van Krieken, and de Jong. *Statistical analysis:* van Oijen and de Jong. *Obtained funding:* van de Kerkhof. *Administrative, technical, or material support:* Berends, Drenth, and de Jong. *Study supervision:* van de Kerkhof, Drenth, van Krieken, and de Jong.
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4
Chapter

Management, efficacy and safety of biologicals

This chapter was based on the following publication:

M.A.M.Berends, R.J.B. Driessen, A.M.G. Langewouters, J.B. Boezeman, P.C.M. van de Kerkhof, E.M.G.J. de Jong
Etanercept and efalizumab treatment for high need psoriasis. Effects and side effects in a prospective cohort study in outpatient clinical practice. *Journal of Dermatological Treatment 2007; 18(2): 76-83.*

Management, efficacy and safety of biologicals

Chapter 4 is about the management, safety and efficacy of the treatment of patients with moderate to severe psoriasis with etanercept and efalizumab.
Many patients are challenged to manage their disease for decades. However, the suitability of classical systemic treatments as continuous long-term therapeutic options is questionable and despite the efforts to optimize available treatments by various approaches (combination, rotation, sequential, intermittent) in some 'high-need' patients, disease control is insufficient In those 'high-need' patients, the biologicals may be able to provide a more consistent control of symptoms with conserveration of a good safety profile.
So far our information is restricted to clinical trials. However, such data do not reflect the everyday situation in the dermatologists' office. In addition, patients categories in trials do not reflect the high-need population for whom those biologicals are indicated for. It is therefore of great importance that patients treated with these new agents are monitored carefully and systematically to optimize the treatment of psoriasis patients with biologicals. As treatment with biologicals is restricted to high need patients with many years previous history of serious disease, long-term treatment with biologicals is anticipated for. Therefore, long-term consistent monitoring and patient databases are very important.
To evaluate the efficacy, safety and adverse events of etanercept and efalizumab in daily practice, a prospective cohort study was carried out between February 2005 and March 2006. The cohort represented a high-need population. To obtain data a special consulting-hours was set up where psoriasis patients with biological treatment were seen on a regular basis and monitored by laboratory results, and special forms were made so reported side effects, concomitant medications, PASI-scores and adverse events could easily be written down. Results were put into a specialized database. These results provide us new and important information about safety, adverse events and efficacy in high-need psoriasis patients in clinical practice and help us to improve management of the treatment of psoriasis patients with these agents.

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Etanercept and efalizumab treatment for high-need psoriasis. Effects and side effects in a prospective cohort study in outpatient clinical practice

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Abstract
Background: Since the beginning of 2005, etanercept and efalizumab are officially registered and reimbursed for the treatment of recalcitrant psoriasis in The Netherlands. *Objective*: The evaluation of the efficacy, safety and adverse events of etanercept and efalizumab treatment in daily practice. *Methods*: A prospective cohort study was carried out for patients treated with etanercept or efalizumab between February 2005 and March 2006. *Results*: Over the past 13 months 45 individuals were treated with etanercept and 17 subjects were treated with efalizumab. The cohort represented a high-need population. At week 12, 82% of the subjects treated with 2×50 mg etanercept/week and 71% of the subjects treated with 2×25 mg etanercept/week reached a PASI-50. Efficacy of etanercept treatment was comparable to the results of clinical trials. For efalizumab, efficacy in responding patients was also comparable to clinical trial data, but the percentage of dropouts was substantial. During biologic treatment, safety was preserved and mainly mild adverse events were reported. *Conclusion*: Etanercept and efalizumab are effective and safe treatments of psoriasis, even in a high-need population. Etanercept was able to sustain the clinical improvement throughout 24 weeks, whereas efalizumab was not in 47% of subjects.

Key words: *Clinical practice, efalizumab, etanercept, high-need psoriasis*

Introduction

Etanercept and efalizumab belong to the newest antipsoriatic therapies, known as biologicals. These drugs became of particular interest for the treatment of psoriasis after discovering the high potential combined with assumed fewer side effects than regular systemic antipsoriatic therapies.

In September 2004, the European Union approved etanercept and efalizumab for the treatment of adult patients with moderate-to-severe plaque psoriasis who failed to respond to, or have a contraindication to, or are intolerant of other systemic therapies (1). In The Netherlands, these pharmaceuticals have been reimbursed by health insurance since the beginning of 2005. Etanercept binds specifically to TNF-α and blocks its interaction with cell surface TNF-α receptors (2). Efalizumab binds to human CD11a, the α-subunit of leukocyte function antigen-1 (LFA-1), thereby inhibiting the adhesion of leukocytes to other cell types (3).

Many well-designed trials have been performed to study the efficacy of biologicals in considerable numbers of patients. The use in daily practice, however, is different from the setting in which trials are conducted. From February 2005 until March 2006, we started with biologic therapy in 62 individuals with recalcitrant psoriasis in our outpatient clinic.

In this report we describe the first year of experience in treating patients in daily clinical practice with moderate-to-severe plaque psoriasis with etanercept and efalizumab. Evaluating the efficacy and safety of biologic agents in clinical practice rather than in clinical trials provides relevant additional information about these new therapeutic strategies in the day-to-day care of psoriasis.

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Patients and methods

Patients

Data were collected prospectively, using a standard form at each visit for all patients treated with etanercept or efalizumab between February 2005 and March 2006. Patients came into consideration for one of these treatments if they had failed to respond to phototherapy, methotrexate and cyclosporin in the past, or if they had a contraindication to, or were intolerant of one of these treatments. At the same time, patients had to have a minimum psoriasis area and severity index (PASI) of 10 at the time of screening, as stated in the guidelines of the Dutch Society of Dermatology and Venereology.

Charts were reviewed for demographics and baseline characteristics, including age, sex, existence of psoriatic arthritis, duration of psoriasis, baseline PASI, previous dermatological treatments and the number of concomitant non-dermatological drugs.

Protocol

Before treatment, a chest X-ray and a Mantoux skin test were performed to exclude tuberculosis. Patients were treated with etanercept or efalizumab, depending on the physician's preference. For etanercept, two dosing regimens were used randomly, either 50 mg subcutaneously (s.c.) twice weekly for 12 weeks, followed by 25 mg s.c. twice weekly for 12 weeks (dosage schedule 1), or 25 mg s.c. twice weekly for 24 weeks (dosage schedule 2). After 24 weeks, patients interrupted etanercept treatment for an indefinite period according to the approved EMEA label. Efalizumab was given in a single conditioning dose of 0.7 mg/kg s.c., followed by 1 mg/kg weekly (up to a maximum single dose of 200 mg).

Contraindications for etanercept treatment were an active infection or increased susceptibility for infections (including immunocompromised individuals), a history of tuberculosis, the existence of a demyelinating disease and pregnancy. Relative contraindications were the existence of cardiac decompensation, a blood dyscrasia, a malignancy in recent history, the presence of an anti-nuclear antibody (ANA) positive autoimmune disease or chronic exposition to actinic radiation in the past.

Contraindications for therapy with efalizumab were the presence of pustular, guttate or erythrodermic psoriasis during screening, a previous malignancy (basal cell carcinomas excluded), active infection or increased susceptibility for infections (including immunocompromised individuals), active tuberculosis, and pregnancy. Relative contraindications were the existence of leukocytosis, lymphocytosis or thrombocytopenia, the presence of an ANA positive autoimmune disease or chronic exposition to actinic radiation in the past.

Patients were allowed to use topical dermatological therapies during biologic treatment. An effort was made to confine the use of concomitant systemic dermatological therapies in cases of unsatisfactory effectiveness of etanercept or efalizumab. Termination of other non-dermatological drugs was found unnecessary.

Visits were scheduled every 4 weeks during the first 12 weeks, every 6 weeks until week 24 and every 12 weeks thereafter. At each visit, the PASI and adverse events were documented. Furthermore, laboratory tests were conducted, including haematological analysis, serum chemistry, urinalysis and ANA. After 12 weeks of therapy, the treatment protocol required an improvement in PASI of at least 50% for both etanercept and efalizumab patients. Patients who did not meet this criterion were excluded from therapy according to the reimbursement guidelines. In some of these cases, treatment with the other available biologic agent was started thereafter. The administration of etanercept or efalizumab was discontinued if patients developed a serious infection; therapy was restarted after recovery. Likewise, therapy was interrupted in cases of elective surgical procedures.

Analysis

Data from charts and forms were transported to a database and analysed to define treatment efficacy by means of PASI, with primary efficacy endpoints including the achievement of an improvement in PASI relative to a baseline of at least 50% or 75% (PASI-50 and PASI-75, respectively). Efficacy analysis of the first 12 weeks of treatment was made according to the intention-to-treat principle. Missing PASI at given time points were imputed using the last observation carried forward (LOCF). Because there were large differences in the follow-up periods of subjects, efficacy of the next 12 weeks was measured by means of a per protocol analysis. Reported adverse events and abnormal laboratory values were summarized. If patients received both etanercept and efalizumab treatment, they were considered two individual subjects and chart analysis was performed twice. Multiple occurrences of the same adverse event in a single subject were counted once.

Results

Demographics

Over the past 13 months 45 subjects were treated with etanercept, of whom 28 received a dose of 50 mg s.c. twice weekly for 12 weeks, followed by 25 mg s.c. twice weekly for 12 weeks (dosage schedule 1) and 17 received a dose of 25 mg s.c. twice weekly for 24 weeks (dosage schedule 2).

Seventeen subjects were treated with efalizumab. Overall, 55% were male, the mean age was 50.9 years and the mean duration of psoriasis was 21.6 years. Out of all the subjects, 18 suffered from psoriatic arthritis. The mean PASI at baseline was 19.8. Patients had previously received four to 10 different dermatological therapies, with an average of 6.7 treatments per patient. The mean number of systemic therapies that patients had used before the start of biologic treatment was 3.3, indicating a high-need population. Some patients had even used biologic agents in the past, mostly in the context of clinical trials. These included etanercept, alefacept, efalizumab, infliximab and onercept. The mean number of concomitant non-dermatological drugs was 2.2 (Table I).

Patients were treated for various periods. The overall mean duration of treatment was 26 weeks (interruptions after 24 weeks or for other reasons excluded), with a range of 5–46 weeks in the etanercept dosage schedule 1 group, 11–49 weeks in the etanercept dosage schedule 2 group and 6–32 weeks in the efalizumab-treated group.

Efficacy

Twenty-three subjects (82%) in the etanercept dosage schedule 1 group, 12 subjects (71%) in the etanercept dosage schedule 2 group and 10 subjects (59%) in the efalizumab group achieved a PASI-50 at week 12. At the same time, respectively, 39%, 24% and 6% achieved a PASI-75 (Table II).

For 14 subjects in the etanercept dosage schedule 1 cohort, 14 subjects in the dosage schedule 2 cohort and four subjects in the efalizumab cohort, PASI at 24 weeks of treatment were available. After per protocol analysis, data revealed the achievement of a PASI-50 in respectively 71%, 79% and 100% of these subjects. Nevertheless, these efficacy percentages are calculated by dividing on the remaining number of subjects at week 24. In the efalizumab cohort, the dropout rate due to lack of efficacy at

Table I. Baseline demographic data and disease characteristics.

	Etanercept			
	Dosage schedule 1	Dosage schedule 2	Efalizumab	All
Age, years				
Range	28–71	39–42	27–71	27–71
Mean (±SEM)	49.6 (1.7)	52.9 (2.3)	51.1 (3.2)	50.9 (1.3)
Gender, no. (%)				
Male	16 (57)	9 (53)	9 (53)	34 (55)
Female	12 (43)	8 (47)	8 (47)	28 (45)
Psoriasis, no. (%)				
With psoriatic arthritis	11 (39)	2 (12)	5 (29)	18 (29)
Without psoriatic arthritis	17 (61)	15 (88)	12 (71)	44 (71)
Duration of psoriasis (years)				
Range	3–42	5–54	3–40	3–54
Mean (±SEM)	20.7 (1.9)	28.0 (3.1)	16.8 (2.5)	21.6 (1.5)
Baseline PASI score				
Mean (±SEM)	25.1 (2.2)	16.3 (1.2)	14.7 (1.3)	19.8 (1.2)
Previous treatments (no.)				
Range, total	4–10	4–9	4–9	4–10
Range, systemic treatments	1–6	2–6	2–5	1–6
Mean, total (±SEM)	6.8 (0.3)	6.4 (0.3)	6.8 (0.3)	6.7 (0.2)
Mean, systemic treatments (±SEM)	3.5 (0.2)	3.0 (0.2)	3.4 (0.3)	3.3 (0.1)
Types of previous treatments used, no. of patients (%)				
Topical steroids/ vitamin D analogues	28 (100)	17 (100)	17 (100)	62 (100)
Dithranol	19 (68)	12 (71)	14 (82)	45 (73)
Methotrexate	28 (100)	17 (100)	16 (94)	61 (98)
UVB	25 (89)	14 (82)	15 (88)	54 (87)
PUVA	21 (75)	14 (82)	11 (65)	46 (74)
Acitretin	20 (71)	12 (71)	13 (76)	45 (73)
Cyclosporin A	21 (75)	9 (53)	13 (76)	43 (69)
Fumaric acid	15 (54)	6 (35)	7 (41)	28 (45)
Etanercept	2 (7)	3 (18)	3 (18)	8 (13)
Alefacept	2 (7)	2 (12)	4 (24)	8 (13)
Efalizumab	6 (21)	0 (0)	0 (0)	6 (10)
Infliximab	2 (7)	0 (0)	2 (12)	4 (6)
Onercept	1 (4)	2 (12)	0 (0)	3 (5)
Concomitant non-dermatological drugs, no.				
Range	0–9	0–6	0–9	0–9
Mean (±SEM)	2.5 (0.5)	1.7 (0.4)	2.1 (0.6)	2.2 (0.3)

SEM=standard error of the mean. Dosage schedule 1: 50 mg twice weekly for 12 weeks, followed by 25 mg twice weekly. Dosage schedule 2: 25 mg twice weekly.

Table II. Efficacy results after 12 weeks of biologic therapy (intention to treat analysis).

	Etanercept			
	Dosage schedule 1	Dosage schedule 2	Efalizumab	All
No. of patients ITT	28	17	17	62
No. of dropouts due to lack of efficacy	1 (4)	2 (12)	5 (29)	8 (13)
⩾50% improvement in PASI, no. (%)	23 (82)	12 (71)	10 (59)	45 (73)
⩾75% improvement in PASI, no. (%)	11 (39)	4 (24)	1 (6)	16 (26)
<50% improvement in PASI, no. (%)	4 (14)	3 (18)	2 (12)	9 (15)

ITT=intended to treat with biologic therapy; PASI=psoriasis area and severity index. Dosage schedule 1: 50 mg twice weekly for 12 weeks, followed by 25 mg twice weekly. Dosage schedule 2: 25 mg twice weekly. Percentages are calculated by dividing on the number of subjects intended to treat.

week 24 was high, i.e. eight subjects (47%) (Table III).

For 18 of all the etanercept-treated subjects, a PASI beyond 24 weeks was available. Analysis of the effect of treatment interruption, after 24 weeks to conform to EMEA guidelines or due to other reasons, on PASI in this group showed that these patients encountered a mean increase in PASI of 2.97 (SEM $\pm$ 1.07) during interruption, with a mean increase of 0.65 per week (Figures 1 and 2). Currently, 12 of these subjects have reached a PASI-50 and 8 have achieved a PASI-75. The only subject in the efalizumab group, who was treated beyond 24 weeks, has currently achieved an improvement in PASI relative to baseline of 69.5%.

Despite a considerable reduction in the severity of disease in many patients, the effects of biologic therapy were unsatisfactory several times. In these cases, concomitant use of other antipsoriatic therapies was necessary. In 91% of all etanercept-treated patients and 82% of all efalizumab-treated patients, topical corticosteroids or vitamin D analogues were used in addition to biologic treatment. Other concomitant therapies included methotrexate, dithranol, acitretin and fumaric acid. Five subjects in the etanercept dosage schedule 1 cohort, one subject in the etanercept dosage schedule 2 cohort and one patient in the efalizumab cohort used one of these therapies.

Side effects

In general, etanercept and efalizumab treatment was well tolerated, and mainly mild adverse events were reported. The most common side effects reported (with an overall incidence of more than 20%) were upper respiratory infections, flu-like symptoms and gastrointestinal symptoms. No subjects were diagnosed with tuberculosis, although, after the marker study period, we discovered three patients with possible latent tuberculosis who had to be prophylactically treated with isoniazid. Malignancies were

Table III. Efficacy results after 18 and 24 weeks of biologic therapy (per protocol analysis).

	Etanercept			
	Dosage schedule 1	Dosage schedule 2	Efalizumab	All
No. of patients at baseline	28	17	17	62
No. of patients with unfinished follow-up				
At week 18	10	1	6	17
At week 24	13	0	5	18
No. of dropouts due to lack of efficacy				
At week 18	1	2	6	9
At week 24	1	3	8	12
Remaining no. of patients				
At week 18	17	14	5	36
At week 24	14	14	4	32
⩾50% improvement in PASI, no. (%)				
At week 18	15 (88)	11 (79)	5 (100)	31 (86)
At week 24	10 (71)	11 (79)	4 (100)	25 (78)
⩾75% improvement in PASI, no. (%)				
At week 18	8 (47)	9 (64)	1 (20)	18 (50)
At week 24	7 (50)	8 (57)	1 (25)	16 (50)
<50% improvement in PASI, no. (%)				
At week 18	2 (12)	3 (21)	0 (0)	5 (14)
At week 24	4 (29)	3 (21)	0 (0)	7 (22)

Dosage schedule 1: 50 mg twice weekly for 12 weeks, followed by 25 mg twice weekly. Dosage schedule 2: 25 mg twice weekly. Efficacy was measured by means of a per protocol analysis. Percentages are calculated by dividing on the remaining number of subjects at week 18 and 24.

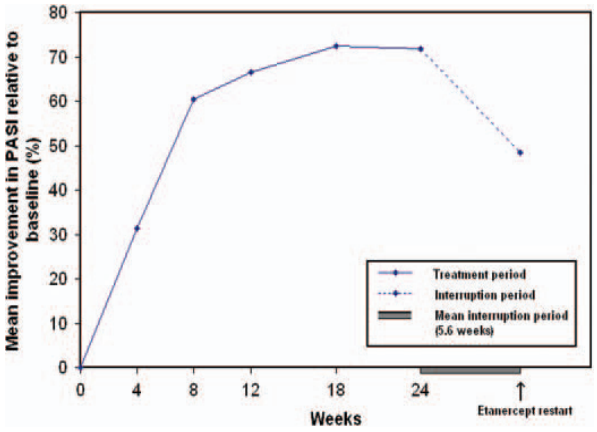

Figure 1. Effect of etanercept interruption at week 24 in 18 subjects.

found in two subjects. One 48-year-old man was diagnosed with an oesophageal carcinoma, as well as with three squamous cell carcinomas and Bowen's disease during etanercept therapy. Another etanercept-treated patient developed a basal cell carcinoma and a squamous cell carcinoma. Both patients were treated with phototherapy in the past.

Infections were reported 38 times. These included upper and lower respiratory infections, skin infections, eye infections, urinary tract infections and oral infections. Eye infections were seen four times in the etanercept group only and none in the efalizumab group (Table IV).

Etanercept therapy was interrupted 12 times: seven times because of upper or lower respiratory infections, twice because of flu-like symptoms and three times due to an elective surgical procedure. Efalizumab treatment was only interrupted once, due to the scheduling of surgery. Five patients required hospital admission: once because of severe arthralgia in combination with a high increase in the C-reactive protein (118 mg/l) value and four times through severe exacerbation of psoriasis. The latter were seen after abrupt discontinuation of other systemic antipsoriatic treatments before the start of biologic therapy, and consecutively had a poor response on biologic treatment; after the occurrence of infection during biologic therapy, that caused discontinuation of biologic therapy; or both. Four of the subjects, who needed admission to hospital, were treated with efalizumab at that time, including the patient with arthralgia.

Seven efalizumab-treated patients (41%) reported changes in the morphologic pattern of psoriasis since

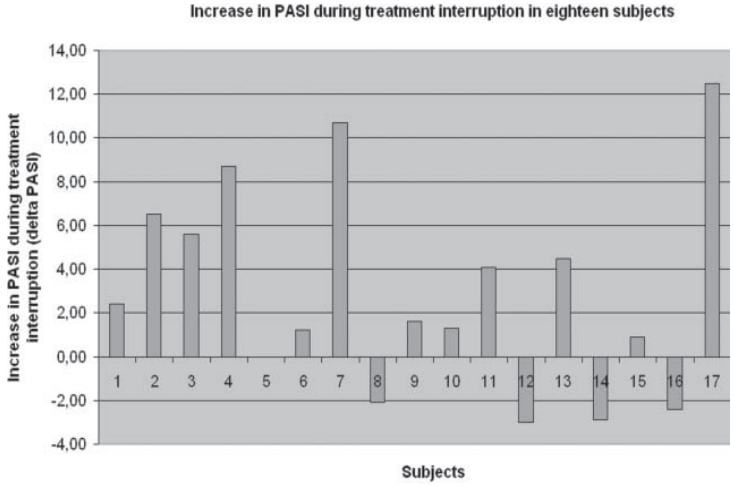

Figure 2. Increase in PASI during treatment interruption in 18 subjects.

Table IV. Adverse events.

	Etanercept			
	Dosage schedule 1	Dosage schedule 2	Efalizumab	All
Infections, no. (%)				
Upper respiratory infections	13 (46)	6 (35)	2 (12)	21 (34)
Lower respiratory infections	2 (7)	2 (12)	1 (6)	5 (8)
Skin infections	0 (0)	2 (12)	2 (12)	4 (6)
Eye infections[a]	0 (0)	4 (24)	0 (0)	4 (6)
Urinary tract infections	1 (4)	2 (12)	0 (0)	3 (5)
Oral infections	0 (0)	1 (6)	0 (0)	1 (2)
(Pre)malignancies, no. (%)				
Actinic keratosis	1 (4)	1 (6)	1 (6)	3 (5)
Squamous cell carcinoma	2 (7)	0 (0)	0 (0)	2 (3)
Bowen's disease	1 (4)	0 (0)	0 (0)	1 (2)
Basal cell carcinoma	1 (4)	0 (0)	0 (0)	1 (2)
Esophageal carcinoma	1 (4)	0 (0)	0 (0)	1 (2)
Skin reactions, no. (%)				
Skin reactions[b]	6 (21)	4 (24)	0 (0)	10 (16)
Pruritus	2 (7)	3 (18)	4 (24)	9 (15)
Injection site reactions	5 (18)	1 (6)	1 (6)	7 (11)
Edema	1 (4)	1 (6)	1 (6)	3 (5)
Hair loss	1 (4)	0 (0)	1 (6)	2 (3)
Miscellaneous, no. (%)				
Flu-like symptoms[c]	5 (18)	4 (24)	6 (35)	15 (24)
Gastrointestinal symptoms[d]	5 (18)	4 (24)	5 (29)	14 (23)
Arthralgia	3 (11)	6 (35)	2 (12)	11 (18)
Headache	1 (4)	4 (24)	7 (41)	12 (19)
Otalgia	1 (4)	2 (12)	0 (0)	3 (5)
Eye irritation	1 (4)	1 (6)	0 (0)	2 (3)
Hypoglycaemias	0 (0)	1 (6)	0 (0)	1 (2)
Dyspnea	0 (0)	0 (0)	1 (6)	1 (2)
Epistaxis	0 (0)	0 (0)	1 (6)	1 (2)
Hospital admission	0 (0)	1 (6)	4 (24)	5 (8)
Any	23 (82)	17 (100)	14 (82)	54 (87)

Dosage schedule 1: 50 mg twice weekly for 12 weeks, followed by 25 mg twice weekly. Dosage schedule 2: 25 mg twice weekly. Multiple occurrences of the same event in a single subject were counted once in the overall incidence. [a]Such as blepharitis, conjunctivitis; [b]such as drug eruption, prurigo nodularis, photodermatosis, mollusca contagiosa, mycosis, urticaria, eczema, pseudofollicultis barbae; [c]such as myalgia, fatigue, chills, sweating; [d]such as nausea, vomiting, diarrhea, abdominal pain, loss of appetite, rectal bleeding.

the start of treatment. Such morphologic changes did not occur in the etanercept cohort.

Routine laboratory monitoring did reveal leukocytosis (white blood cell count $> 11 \times 10^9$/l) in 23 patients at one or more moments during therapy. Twelve of these patients were in the etanercept cohort (27%) and 11 patients were in the efalizumab group (65%). Fourteen times leukocytosis was combined with an increased CRP (CRP > 10 mg/l): six times in the etanercept group and eight times in the efalizumab group. However, only seven patients reported clinical signs of infection (etanercept: $n=4$, efalizumab: $n=3$), principally diagnosed as mild upper respiratory infections and skin infections. Thrombocytopenia was seen three times, one in every treatment group. No other notable changes in laboratory markers were found during therapy.

Current status

At the beginning of March 2006, 48 subjects were still on biologic treatment, including three subjects who had temporarily discontinued therapy. Of the efalizumab cohort, 47% discontinued therapy because of lack of efficacy. On the contrary, only six subjects (13%) terminated etanercept treatment. One of these six subjects (i.e. the patient with the oesophageal carcinoma and squamous cell carcinomas) discontinued therapy because of these adverse events, diagnosed at week 44.

Discussion

Our aim was to evaluate the efficacy, safety and adverse events of etanercept and efalizumab treatment in the outpatient clinic. Data were obtained from a 1-year follow up of 62 patients. The cohort represented a high- need population, concerning the treatment of psoriasis.

A total of 82% of the subjects in the etanercept dosage schedule 1 group and 71% of the subjects in the etanercept dosage schedule 2 group achieved a PASI-50 at week 12; 39% and 24%, respectively, achieved a PASI-75 at this point. These efficacy data are comparable with the results of several clinical

trials (4–6). During the next 12 weeks, the efficacy of etanercept treatment remained stable.

Efalizumab efficacy data were much less satisfying. A total of 59% of all subjects achieved a PASI-50, but only 6% achieved a PASI-75 at week 12. Furthermore, after 24 treatment weeks, eight of the 17 efalizumab-treated patients discontinued therapy because of lack of efficacy. In addition, four subjects in this cohort needed hospital admission during therapy. This is in contrast with efalizumab clinical trial data, which show significant PASI improvements in large numbers of patients (7–11).

It has to be kept in mind that these data are presumably influenced by the use of concomitant antipsoriatic therapies. More than 80% of all subjects needed concomitant use of topical steroids or vitamin D analogues, and in seven of all the subjects, the use of other systemic antipsoriatic treatments or dithranol was even necessary. It is an important goal to investigate further the effect of combining biologicals with other antipsoriatic treatments. Combining etanercept with methotrexate has already been found to be more effective in the treatment of rheumatoid arthritis than etanercept monotherapy (12).

Abrupt cessation of other systemic antipsoriatic treatments before starting with biologicals could possibly influence the efficacy of these drugs in a negative manner. Likewise, interruption of etanercept treatment after 24 weeks, as we did according to the EMEA label, appears to elicit a substantial fall in treatment benefits. Taking this into account, we recommend a gradual tapering of systemic antipsoriatic treatments before, or partially overlapping, biologic therapy. In addition, continuing treatment after 24 weeks instead of interrupting therapy at that point would be of benefit to the patient.

Both etanercept and efalizumab were well tolerated. Fifty-four patients reported one or more side effects, but those were mainly mild. The most frequently reported side effects were upper respiratory infections, flu-like symptoms and gastrointestinal symptoms. Eye infections were only seen in the etanercept cohort, as well as eye irritation. Recently, Taban et al. accomplished a literature review about inflammatory eye disease associated with etanercept therapy, and found that ocular inflammation is a potential adverse event following the use of etanercept (13). Of significance as well are the nine subjects in the etanercept cohort reporting arthralgias. Only two of these subjects suffered from psoriatic arthritis. Physical examination of the other subjects by a rheumatologist did not reveal a significant arthritis.

Changes in the morphologic pattern of psoriasis since the start of treatment were reported by 41% of the efalizumab-treated patients. In some cases this meant the manifestation of psoriasis in regions that were not affected earlier; in other cases, the plaque-type psoriasis appeared to change in another type, such as guttate or pustular psoriasis. This phenomenon was also seen in other trials, although an incidence of 3.2% was mentioned (14).

As leukocytosis is often seen during treatment with efalizumab, it should not be used as an infection parameter. Therefore, to monitor infections, physicians should pay better attention to the clinical symptoms of infection, reported by patients. Furthermore, as latent tuberculosis was found three times in our cohort, we recommend performing tuberculosis screening in all patients who are candidates for biologic therapy.

In conclusion, prospective cohort monitoring of high-need psoriasis patients on systemic treatments, especially on biologicals, is worthwhile. Information about treatment with these new drugs in daily clinical practice is important for adjusting treatment schedules and guidelines.

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5
Chapter

General Discussion

5.1 General focus

The studies carried out in this thesis were designed to optimize the available antipsoriatic treatments with respect to safe disease control. The following aims were defined:

1. To understand the improved efficacy of a combination therapy of calcipotriol and betamethasone dipropionate in terms of effects on T-cell subsets and epidermal proliferation and differentiation.
2. To define the degree of Methotrexate-induced hepatotoxicity and to develop new strategies for monitoring this hepatotoxicity
3. To set up a special unit and a database for high-need psoriasis patients treated with biologicals to provide best possible care and to evaluate the efficacy and safety of these relatively new treatments in daily practice.

In the discussion we will further integrate the observations in the present thesis with available data found in the literature. Further we will propose some suggestions for modification of the available guidelines. Finally we will propose some future directions.

Aim I: To understand the improved efficacy of a combination therapy in terms of effects on T-cell subsets and epidermal proliferation and differentiation.

Chapter 2 compared the clinical and histological effects of betamethasone diproprionate (topical corticosteroid) and calcipotriol (vitamin D_3 derivative) as monotherapy and as combination. It is concluded that the action spectra of calcipotriol and betamethasone on the psoriatic plaque are different and that the combination has effects on T-cell subsets, beyond the addition of the effects of mono therapies with major reductions of all studied T-cell subsets in epidermis and dermis (CD4, CD8, CD25, CD45RO, CD45RA, CD94, CD161 and CD2). Combination therapy enables a reduction of the required amount of both monotherapies thereby reducing the risk of side effects. Reduction of side effects in combination with conservation of effect can indeed be regarded as optimization of a therapy.

Since 2003 the two-compound product, Dovobet®/Daivobet®/Taclonet®, containing calcipotriol 50 µg/g plus betamethasone dipropionate 0.5 mg/g is available on the market for the treatment of psoriasis. Clinical efficacy has been proved to be significantly better than monotherapy with the above named individual ingredients with at least similar or even significantly better safety profile for the combination therapy[1-3]. Safety data of up to a 52-week treatment period are available[4]. The advised treatment scheme is once daily application for 4 weeks and then intermittently as needed[3].

Topical agents can be combined with each other, with phototherapy and systemic treatments. Despite of decades of experience with clinical usage of topical agents, evidence-based data for efficacy and safety are limited. Furthermore, little information is available on the combination of topical treatments with systemic agents. The most extensive available evidence-based data until now are on the vitamin D_3 analogue calcipotriol as monotherapy or in combination with another topical or systemic therapy and the above described two-compound combination.

To evaluate and optimize the treatment of psoriasis in order to maintain clinical efficacy with minimal side effects and to define an optimal appropriate therapeutic ladder, well-designed studies with topical treatments and combination therapies are required to support evidence-based treatment guidelines.

Aim II: To define the degree of Methotrexate-induced hepatotoxicity and to develop new strategies for monitoring this hepatotoxicity.

Chapter 3 is about MTX hepatotoxicity.

MTX is a folic acid antagonist and there is substantial evidence that it acts by inhibiting DNA synthesis. Possibly, as a consequence it possesses potent anti-inflammatory effects on T-cell mediated immune responses as it inhibits proliferation and induces apoptosis in activated T-cells and blocks the abnormal rapid epidermal cell proliferation, both responsible for the characteristic skin lesions in psoriasis[5].

MTX plays a central role in the treatment of severe psoriasis in Europe[6]. This role is strengthened by the fact that reimbursement of the expensive biologicals in Europe is restricted to 'high-need' psoriasis patients. 'High-need' psoriasis patients may be defined using criteria that include disease severity and resistance and/or intolerance to a number of conventional therapies[7, 8], like MTX. Furthermore, in the future, MTX will more and more be used in combination with one of these biologicals.

However, long-term MTX therapy is associated with some serious adverse reactions as myelosuppression, interstitial pneumonitis and hepotoxicity[9]. However, the reported frequency of MTX-induced liver fibrosis and cirrhosis in psoriasis patients varies widely in different studies[10-12]. High numbers of prevalence of liver fibrosis and cirrhosis and uncertainty about a delineated effect led to dermatologic guidelines that stipulate monitoring periodically after every 1500 mg cumulative dose of MTX[13]. Until now the gold standard in the assessment of histological changes is a liver biopsy[14]. The need for a liver biopsy impedes the prescription of MTX.

The results of a Dutch survey, conducted among dermatologists and residents in dermatology, revealed that the need for liver biopsies in combination with the frequent check-ups and the lack of

consensus between rheumatologists, hepatologists and dermatologists restrict the adherence to the guideline of the Dutch Society of Dermatology and Venereology with respect to the treatment with MTX for severe chronic plaque psoriasis. Since MTX retains a central role in the treatment of psoriasis in Europe and because of the resemblance of the Dutch guideline to the international guidelines[13], our results may, at least to some extent, be applicable to other countries.

The obtained knowledge that the liver biopsy is one of the reasons which impede the prescription of MTX in psoriasis patients by dermatologists combined with the great variation in prevalence of MTX-induced liver injury in psoriasis suggested that reconsideration of our current monitoring strategies for patients on MTX might be warranted.

Therefore, in a following study the prevalence and development of liver injury in MTX treated psoriasis was evaluated. From this study it could be concluded that MTX-related liver injury is less frequent than previously thought and mostly occurs at a cumulative dose of < 6000 mg, running a benign course. Diabetes and overweight are significantly correlated with liver injury. These results are confirmed by a recent study[15]. These results suggest that monitoring for MTX-induced liver fibrosis in psoriasis patients with risk factors, as diabetes and overweight should be continued according to the guidelines. However, for patients without these risk factors, the guideline might be reconsidered.

The reason that the application of a liver biopsy, amongst other things, restricts the adherence to the guideline is that it is complicated by some important limitations. These limitations include the risk for complications caused by the procedure: such as pain and localized bleeding which are more frequent issues and pneumothorax, haemothorax, bile peritonitis, haemobilia, and inadvertent puncture of the kidney or intestine which occur less often[16, 17]. The complication risk of a liver biopsy is approximately 1-2%, with a 0.01-0.1% risk of mortality[18]. Other limitations of a liver biopsy are the sampling error, which according to some studies can approach 30%, intra- and inter-pathologist inconsistency and discontinuous and semi-quantitative histological scoring systems[13, 14, 16, 19-23]. Finally, a liver biopsy is an expensive procedure, requiring hospitalization.

Therefore there is a pressing need for non-invasive, reliable alternative methods to monitor MTX-induced liver injury in psoriasis patients. It has been shown that liver fibrosis can develop despite normal serum liver values and normal ultrasound and radioisotope scans[24]. A promising method for monitoring liver damage might be the aminoterminal propeptide of procollagen type III (PIIINP). Studies have shown that liver biopsies can be avoided when serial PIIINP (once per 3 months) are continuously normal[18, 25-27]. Furthermore, in Hepatitis C patients two other, non-invasive methods, the Fibroscan® and Fibrotest, have been tested and found promising in monitoring liver damage[21, 23, 28-32].

We performed a pilot study to evaluate the accuracy and feasibility of the Fibroscan® and Fibrotest to assess MTX-induced hepatic fibrosis in psoriasis patients. The promising results suggest that a combination of Fibroscan® and Fibrotest should prospectively be evaluated in monitoring and detecting significant MTX-induced liver fibrosis in psoriasis patients. Also more prospective studies with serial PIIINP and liver biopsies as reference should be performed.

As described above, another limitation of a liver biopsy is its shortage of a good histological scoring system. MTX-associated liver injury in a liver biopsy is graded by pathologists according to the Roenigk classification. However, the Roenigk classification is subjective and insensitive to small changes, particularly when assessing fibrosis lumping all biopsies with more than minimal fibrosis as advanced fibrosis[33-35]. Furthermore, this classification has never been validated and no literature is known about the inter-observer reliability. Since the assessment of liver damage is essential in the management of psoriasis patients the results of the Roenigk scoring system should be reproducible with little inter-observer error.

Our study showed that the interobserver variability of the Roenigk score as classification system of liver damage is low. Also, there was a good agreement on biopsies that resulted in a Roenigk grade that necessitated change of clinical management (biopsies with Roenigk 3a, 3b or 4).

A recent study demonstrated preliminary evidence that specific polymorphisms of enzymes involved in folate, pyrimidine and purine metabolism could be useful in predicting clinical response, as in efficacy and toxicity, to methotrexate in psoriasis patients[36].

Taken all these aspects into account the use of MTX in the treatment of psoriasis patients has been optimized. It has become clear that in case of risk factors frequently monitoring for MTX-induced liver damage is indicated. In patients without risk factors, monitoring frequency might be reduced and adapting the guidelines at this point is advised. For the present, monitoring should still be done by liver biopsy, with Roenigk scoring system having a good inter-observer reliability. However, monitoring liver damage in patients without risk factors might be done by a combination of non-invasive alternatives of the liver biopsy in the future.

Aim III: To set up a special unit and a database for high-need psoriasis patients treated with biologicals to provide best possible care and to evaluate the efficacy and safety of these relatively new treatments in daily practice.

Chapter 4 is about the management, safety and efficacy of the treatment of patients with moderate to severe psoriasis with etanercept and efalizumab.

Most data about these agents have been retrieved from clini-

cal trials and only limited long-term data are available. It is therefore of great importance that patients treated with these new agents are monitored carefully and systematically to optimize the treatment of psoriasis patients with biologicals.

To evaluate the efficacy, safety and adverse events of etanercept and efalizumab in daily practice, a prospective cohort study was carried out between February 2005 and March 2006. The cohort represented a high-need population. To obtain data a special consulting-hours was set up where psoriasis patients with biological treatment were seen on a regular basis and monitored by laboratory results, and special forms were made so reported side effects, concomitant medications, PASI-scores and adverse events could easily be recorded. Results were transported to a database and analysed.

From the results it could be concluded that both etanercept and efalizumab are effective and safe treatments for psoriasis, even in a high-need population considering duration of one year. Nevertheless, frequently monitoring remains required.

The etanercept efficacy data of our study are comparable with the results of other clinical trials with 82% and 39% of the patients treated with 50mg twice weekly and 71% and 24% of the patients treated with 25mg twice weekly reaching a PASI-50 and PASI-75 respectively[8, 37-40]. It should be kept in mind however, that these data might have been influenced by the use of concomitant antipsoriatic therapies. More than 80% of patients used concomitant topical steroids or vitamin D analogues, 7 patients used concomitant systemic antipsoriatic treatments or dithranol.

However, efalizumab efficacy data were much less satisfying compared to literature data with only 6% of the patients reaching a PASI-75 at week 12[8, 37, 39, 39, 40]. These results might be explained by the fact that efalizumab was sometimes started in patients with an unstable form of psoriasis, for example after abrupt cessation of other systemic antipsoriatic therapies (MTX or cyclosporine).

Clinical trials have shown, as described in the introduction, that PASI response rates continue to improve after week 12[37]. Our results agree with those data. Furthermore, interruption of etanercept treatment after 24 weeks, as we did according to the EMEA label, appears to elicit a substantial fall in treatment benefits.

In daily practice, patient category indicated for biological treatment appears to be a real 'high-need' one with many comorbidities, comedication and an extended history of previous used antipsoriatic systemic treatments. This difference in patient category between daily practise and clinical trials and limited available long-term data require a strict prospective cohort monitoring.

All the results obtained by our study can lead to an optimization of the use of biologicals in the treatment of patients with psoriasis. These steps are put into recommendations for adaptation of the guideline and future directions to further optimize their usage.

5.2 Recommendations for adaptation of available guidelines

Based on guidelines of the NHG and the Dutch Society of Dermatology and Venereology[41-43] but also based on the information available in international reviews (Bologna) it is evident that a 'stepwise approach' should, whenever possible, start with topical therapy.

Calcipotriol/ betamethasone dipropionate
According to the NHG standard Psoriasis medical treatment should be started with a class III topical corticosteroid once a day for 4 weeks (with a maximum of 100 grams a week), or with calcipotriol twice a day for 8 weeks (with a maximum of 100 grams week). In case this has insufficient effect, combination therapy with calcipotriol application in the morning and a class III topical corticosteroid in the evening is advised. Application of a class IV corticosteroid, once a day for 4 weeks is the next advised step. Dithranol is advised as final step in case none of the above described treatments are effective enough.

Based on our own experience in combination with efficacy and safety data of other studies we propose combination therapy with the two compound product Daivobet®, once a day for 4 weeks as first step in the topical treatment of psoriasis patients.

After those 4 weeks there are a few possible options for management of follow-up treatment:

1. Intervention therapy: restarting Daivobet® therapy in case of a relapse.
2. Intermittent therapy: application of Daivobet® 3 times a week and the rest of the week application of calcipotriol monotherapy.
3. Maintenance therapy: application of calcipotriol monotherapy alone.
4. In case of frequent relapses: Daivobet® application as requested for a long-term period of time.

Methotrexate
In case of psoriasis patients with diabetes and/or overweight treated with MTX, frequent monitoring for liver damage should be done between 1500 mg and 6000 mg cumulative MTX dose according to the guideline.

In the absence of risk factors, we propose less frequent monitoring. Furthermore, in patients without risk factors we would propose monitoring primary by non-invasive alternatives like PIIINP followed by a liver biopsy in case of abnormal values.

Biologicals
In case of the biologicals we propose adjusting the guideline at the following points:

Since PASI response rates have shown to continue to improve after week 12 we suggest an evaluation point of efficacy of biologicals after 6 months instead of week 12.

We propose continuing treatment of etanercept after 24 weeks under strict monitoring instead of interrupting therapy. Furthermore we recommend gradual tapering of systemic antipsoriatic treatments before, or partially overlapping, biological therapy to prevent unstable situations before starting with a biological. Finally we propose less strict criteria for prescribing biological treatment for psoriasis patients. Expected number of requests for biological treatment in 2006 in the Netherlands was in the order of 1200. However, only 933 requests were received, which is a relatively small quota compared to the number of rheumatology requests received. This suggests that our criteria are too strict, leading to a relatively young 'high-need' population, with already extensive history of dermatological treatments with secondary exposure to toxic amounts of drugs.

The following proposals are possible:

1. Patients are directly eligible for treatment with a biological without any required previous treatments.
2. Patients are eligible for treatment with a biological when they have been treated with at least one of the classical systemic treatments.
3. Patients are eligible when they have been treated with at least 2 of the classical systemic therapies.

Since the restricted experience with biologicals in the dermatology and limited available long-term data we propose gradual adaptation of the guideline and liberalize criteria by requiring at least 2 instead of 3 classical systemic therapies before starting treatment with a biological. In rheumatology criteria are already less strict. For example, in case of psoriatic arthritis only a moderate-severe arthritis in combination with previous treatment with MTX is required.

5.3 Future directions

To further evaluate and optimize the treatment of psoriasis in order to maintain clinical efficacy with minimal side effects and to define an optimal and appropriate therapeutic ladder, well-designed long-term comparative studies with topical treatments and combination therapies are required to support evidence-based treatment guidelines.

Furthermore, well-designed prospective studies are required to continue the search for non-invasive alternatives to monitor MTX-induced liver injury. Besides a prospective study, combining Fibrotest and Fibroscan®, a study with amino terminal peptide of type III procollagen (PIIINP) as non-invasive method to detect liver fibrosis and/or cirrhosis would be interesting. PIIINP was studied before and it has

been shown that, when measured levels at regular intervals are consistently normal, liver biopsies can be avoided. Studies about the patients with high PIIINP but normal liver histology are needed to further diminish the number of unnecessary liver biopsies. In addition, a cost-effective analysis comparing costs and effectivity of a liver biopsy and non-invasive alternatives is indicated.

With respect to the use of biologicals for high-need patients in clinical practice it is of importance to continue prospective intensive monitoring for efficacy and safety data. Therefore the use of a well-designed database is of great help and further modifying this database is of great importance.

Since it has been shown that also the biological agents can not afford some patients a continuous control of their symptoms, it is an important goal to further investigate the efficacy and safety of combination of biologicals with other antipsoriatic treatments. Combining etanercept with MTX has already been found to be more effective in the treatment of rheumatoid arthritis than etanercept monotherapy[44].

Optimization of the treatment of psoriasis requires developments in topical, classical systemic and biological treatments to provide a spectrum of options to the individual patient.

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Chapter 6

Summary

6.1 Summary

Psoriasis is a chronic and incurable disease which challenges many patients to manage their disease for decades. A stepwise approach in the treatment of psoriasis is advised by guidelines and international reviews, starting with (1) topical therapy, followed by photo(chemo)therapy and classical systemic therapies (2), followed by biologicals (3).

In this present thesis we aimed to optimize the treatment of psoriasis, reconciling this treatment paradigm and to carry out a study in each of these 3 phases of intervention. The three major aims of this thesis were:

I To understand the improved efficacy of a combination therapy of calcipotriol and betamethasone dipropionate in terms of effects on T-cell subsets and epidermal proliferation and differentiation. (Chapter 2)

II To define the degree of Methotrexate-induced hepatoxicity and to develop new strategies for monitoring this hepatotoxicity. (Chapter 3)

III To set up a special unit and a database for high-need psoriasis patients treated with biologicals to provide best possible care and to evaluate the efficacy and safety of these relatively new treatments in daily practice. (Chapter 4)

In **chapter 1**, at first the focus of this thesis is presented, followed by an overview of the epidemiology, clinical presentation, aetiology, pathogenesis, the available treatments of psoriasis and the limitations of these treatments. Subsequently the aims of these thesis are formulated.

In **chapter 2**, we compared the effect of the combination of the vitamin D_3 analogue calcipotriol and the corticosteroid betamethasone dipropianate and of both monotherapies on psoriasis relevant T cells, in order to understand the rationale of this successful combination. We concluded that the action spectra of calcipotriol and betamethasone on the psoriatic plaque are different and that the combination has effects on T-cell subsets, beyond the addition of the effects of monotherapies.

In **chapter 3** the degree of methotrexate-induced hepatoxicity and new strategies for monitoring this hepatotoxicity were studied.
Results from a Dutch survey showed that, amongst others, the need for biopsies restrict the adherence to the guideline. Methotrexate-induced liver injury in our studied population appears to be less frequent than previously thought and mostly occurs between 1500-6000 mg cumulative dose, nevertheless suggesting a continued need for monitoring liver injury. Other conclusions were that diabetes mellitus and

overweight are risk factors for developing liver damage and that liver enzymes do not correlate with histological findings. From a pilot-study we concluded that the two used non-invasive methods seem to be of benefit in the screening of methotrexate-induced liver damage in psoriasis patients. Finally we concluded that the Roenigk classification in the assessment of liver fibrosis is a reliable scoring system.

In **chapter 4** we described one year experience with high-need psoriasis patients treated with two relatively new treatments, etanercept and efalizumab in daily practice and we evaluated their efficacy and safety in these patients during this year. Data were obtained by setting up a special unit and a database. From the results it could be concluded that both etanercept and efalizumab are effective and safe treatments for psoriasis, even in a high-need population considering duration of one year.

In **chapter 5** the observations in chapters 2-4 are integrated and positioned in the context of recent literature. Moreover, recommendations for adaptations of the existing guidelines are presented and perspectives for future development are provided.

6.2 Samenvatting

Psoriasis is een chronische en ongeneeslijke ziekte en vele patiënten zijn genoodzaakt om tientallen jaren met hun ziekte te moeten leven. De richtlijnen en internationale literatuur adviseren daarom ook een stapsgewijze benadering in de behandeling van psoriasis en, voor zover mogelijk, te beginnen met (1) topicale therapie, gevolgd, indien noodzakelijk, door (2) foto(chemo)therapy en de klassieke systemische behandelinen en uiteindelijk gevolgd door de biologicals (3).

Het doel van dit proefschrift was de behandeling van psoriasis te optimaliseren, rekening houdend met bovenstaand behandelingsparadigma. Hiertoe zijn in elk van de 3 behandelingsfasen, studies verricht. De 3 belangrijkste doelen van dit proefschrift waren:

I Inzicht krijgen in de verbeterde effectiviteit van een combinatie therapie van calcipotriol en betamethason dipropionaat in termen van de effecten op T-cell subsets en epidermale proliferatie en differentiatie. (Hoofdstuk 2)

II Definiëren van de ernst van methotrexaat geinduceerde leverfibrose en het ontwikkelen van alternatieven om het onstaan en de otnwikkeling van deze leverschade aan te kunnen tonen. (Hoofdstuk 3)

III Het opzetten van een speciaal spreekuur en een database voor 'high-need' psoriasis patiënten die behandeld worden met biologicals om op die manier de best verkrijgbare zorg te kunnen leveren en de effectiviteit en veiligheid van deze, relatief nieuwe, middelen te kunnen evalueren in de dagelijkse praktijk. (Hoofdstuk 4)

In **hoofdstuk 1** wordt om te beginnen de focus van dit proefschrift gepresenteerd. Hierna volgt een overzicht van de epidemiologie, de klinische presentatie, aetiologie, pathogenese, beschikbare behandelingen en beperkingen van deze behandelingen voor psoriasis. Tot slot worden de doelstellingen geformuleerd.

In **hoofdstuk 2** vergeleken we het effect van de combinatietherapie calcipotriol (vitamin D_3 analoog) en betamethason dipropionaat en van beide monotherapieen op de, voor psoriasis, relevante T cellen om op die manier de rationale achter deze succesvolle combinatie te ontdekken. We concludeerden dat de werking van calcipotriol en betamethasondipropionaat op de psoriasis plaque, van elkaar verschillen en dat de combinatie een groter effect heeft op de relevante T-cel subsets dan de monotherapieen afzonderlijk.

In **hoofdstuk 3** werd de ernst van Methotrexaat geinduceerde leverfibrose en nieuwe mogelijke strategieen om deze lever schade te monitoren bestudeerd.

Resultaten van een nederlandse enquete lieten zien dat de noodzaak tot het verrichten van een leverbiopt één van de redenen is voor een beperkte adherentie van dermatologen aan de richtlijn "Methotrexaat" van de nederlandse vereniging voor dermatologie en venereologie. Methotrexaat geïnduceerde leverschade bleek in onze populatie minder vaak voor te komen dan in de literatuur en onstaat met name tussen 1500 en 6000 mg cumulatieve dosis Methotrexaat wat duidt op de noodzaak voor de controle op het onstaan en de ontwikkeling van deze leverschade. Verder concludeerden we dat diabetes mellitus en overgewicht risicofactoren zijn voor het ontwikkelen van leverschade en dat leverenzymen niet correleren met histologische bevindingen. De resultaten van een 'pilot'-studie deed ons concluderen dat de twee gebruikte non-invasieve methodes nuttig lijken te zijn bij de screening voor de aanwezigheid van Methotrexaat geïnduceerde signficante lever fibrose in psoriasis patiënten. Tot slot konden we concluderen dat de Roenigk classificatie een betrouwbare score is in de bepaling van aanwezigheid van leverfibrose.

In **hoofdstuk 4** beschreven we ervaringen van de behandeling van 'high-need' patiënten met twee relatief nieuwe middelen, etanercept en efalizumab, in de dagelijkse praktijk. Tevens evalueerden de effectiviteit en veiligheid van deze middelen bij deze patiënten gedurende dat jaar. Om de data te verkrijgen en te analyseren werden er een speciaal 'biologicals' spreekuur en een speciale database opgezet. Uit de resultaten kon geconcludeerd worden dat gedurende dat jaar, zowel etanercept als efalizumab effectieve en veilige behandelingen bleken, zelfs in een 'high-need' populatie.

In **hoofdstuk 5**, de algemene discussie, worden de resultaten van alle onderzoeken geplaatst in de context van de recente literatuur. Verder worden aanbevelingen gedaan voor het aanpassen van de bestaande richtlijnen en worden suggesties gedaan voor verder onderzoek.

Curriculum Vitae

Maartje Adriana Marike Berends werd op 16 april 1977 geboren te Boko, Benin, Afrika. Op drie jarige leeftijd verhuisde zij naar Nederland. Vele verhuizingen volgden waarbij zij haar jeugd doorgebracht in onder andere Gouda, Prinsenbeek, Skien (Noorwegen) en Assen.

Na het VWO eindexamen in 1996 bracht ze een jaar in Brussel door waar ze een studie Tolk-Vertaler Engels-Deens volgde. Hierna verhuisde ze naar Nijmegen waar ze in 1998 haar propedeuse psychologie behaalde.

In september 1998 werd aangevangen met de studie geneeskunde aan de toenmalige Katholieke Universiteit Nijmegen (nu geheten Radboud Universiteit Nijmegen) alwaar in december 2004 het artsexamen behaald werd. Tijdens het onderwijs gedurende het vierde doctoraal jaar ontstond de interesse voor de Dermatologie welke vervolgens werd vergroot door een wetenschappelijke stage en een keuze co-schap Dermatologie.

Na haar artsexamen werd zij aangenomen als junior onderzoeker bij de afdeling Dermatologie aan de Radboud Universiteit Nijmegen onder begeleiding van professor dr. dr. P.C.M. van de Kerkhof, dr. E.M.G.J. de Jong en professor dr. J.P.H. Drenth van de afdeling Maag-, Darm- en Leverziekten. Dit proefschrift is het resultaat van deze onderzoeksperiode.

Sinds maart 2007 is zij in opleiding tot dermatoloog in het UMC St. Radboud te Nijmegen.

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